

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

***** STN Columbus *****
 FILE 'HOME' ENTERED AT 16:36:45 ON 23 SEP 2003
 => file biosis,caba,caplus,embase,japio,lifesci,medline,scisearch,uspatfull
 => e stewart graham/au

E1 39 STEWART GRAEME J/AU
 E2 2 STEWART GRAEME O/AU
 E3 25 --> STEWART GRAHAM/AU
 E4 5 STEWART GRAHAM A/AU
 E5 176 STEWART GRAHAM G/AU
 E6 6 STEWART GRAHAM GEORGE/AU
 E7 6 STEWART GRAHAM J/AU
 E8 21 STEWART GRAHAM R/AU
 E9 1 STEWART GRAHAM ROGER/AU
 E10 1 STEWART GRAHAM T/AU
 E11 1 STEWART GRAHAM TEAL/AU
 E12 2 STEWART GRAHAM W/AU

=> s e3-e9 and mycobact?

L1 17 ("STEWART GRAHAM"/AU OR "STEWART GRAHAM A"/AU OR "STEWART GRAHAM
 G"/AU OR "STEWART GRAHAM GEORGE"/AU OR "STEWART GRAHAM J"/AU
 OR "STEWART GRAHAM R"/AU OR "STEWART GRAHAM ROGER"/AU) AND MYCOB
 ACT?

=> dup rem l1

PROCESSING COMPLETED FOR L1

L2 9 DUP REM L1 (8 DUPLICATES REMOVED)

=> d bib ab 1-

YOU HAVE REQUESTED DATA FROM 9 ANSWERS - CONTINUE? Y/(N):y

L2 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 2002:675871 CAPLUS
 DN 137:200259
 TI Vaccine compositions comprising modified pathogen overexpressing heat
 shock protein for therapeutic intervention in infectious disease
 IN Young, Douglas Brownlie; ***Stewart, Graham Roger*** ; O'Gaora, Peadar
 Caoimhin Eoin
 PA Sequella, Inc., USA
 SO PCT Int. Appl., 59 pp.
 CODEN: PIXXD2
 DT Patent
 LA English
 FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002067982	A2	20020906	WO 2002-US5038	20020220
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2002172685	A1	20021121	US 2002-79136	20020220
PRAI US 2001-269801P	P	20010220		
US 2001-294170P	P	20010529		

AB Methods and compns. for the treatment and prevention of infectious diseases are provided. In particular, efficient vaccines comprising genetically modified pathogens are provided. The vaccines generally comprise ***mycobacterial*** mutants having modified protein prodn. capabilities. In one embodiment, the mutants overexpress heat shock protein. In a specific embodiment, the ***mycobacterial*** mutant overexpresses heat shock proteins 60 and/or 70. Also provided are modified BCG vaccines capable of overexpressing heat shock proteins 60 and/or 70.

L2 ANSWER 2 OF 9 USPTAFULL on STN

AN 2002:307566 USPTAFULL

TI Methods and compositions for therapeutic intervention in infectious disease

IN ***Stewart, Graham*** , Walton-on-Thames, UNITED KINGDOM

O'Gaora, Peadar, London, UNITED KINGDOM

Young, Douglas, Ruislip, UNITED KINGDOM

PI US 2002172685 A1 20021121

AI US 2002-79136 A1 20020220 (10)

PRAI US 2001-269801P 20010220 (60)

US 2001-294170P 20010529 (60)

DT Utility

FS APPLICATION

LREP JOHN S. PRATT, ESQ, KILPATRICK STOCKTON, LLP, 1100 PEACHTREE STREET, SUITE 2800, ATLANTA, GA, 30309

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 15 Drawing Page(s)

LN.CNT 1922

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods and compositions for the treatment and prevention of infectious diseases are provided. In particular, efficient vaccines comprising genetically modified pathogens are provided. The vaccines generally comprise ***mycobacterial*** mutants having modified protein production capabilities. In one embodiment, the mutants overexpress heat shock protein. In a specific embodiment, the ***mycobacterial*** mutant overexpresses heat shock proteins 60 and/or 70. Also provided are modified BCG vaccines capable of overexpressing heat shock proteins 60 and/or 70.

L2 ANSWER 3 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 1

AN 2002:600499 BIOSIS

DN PREV200200600499

TI Dissection of the heat-shock response in ***Mycobacterium*** tuberculosis using mutants and microarrays.

AU ***Stewart, Graham R. (1)*** ; Wernisch, Lorenz; Stabler, Richard; Mangan, Joseph A.; Hinds, Jason; Laing, Ken G.; Young, Douglas B.; Butcher, Philip D.

CS (1) Department of Infectious Diseases and Microbiology, Centre for Molecular Microbiology and Infection, Imperial College of Science Technology and Medicine, London, SW7 2AZ: g.stewart@ic.ac.uk UK

SO Microbiology (Reading), (October, 2002) Vol. 148, No. 10, pp. 3129-3138. <http://mic.sgmjournals.org>. print. ISSN: 1350-0872.

DT Article

LA English

AB Regulation of the expression of heat-shock proteins plays an important role in the pathogenesis of ***Mycobacterium*** tuberculosis. The heat-shock response of bacteria involves genome-wide changes in gene expression. A combination of targeted mutagenesis and whole-genome expression profiling was used to characterize transcription factors responsible for control of genes encoding the major heat-shock proteins of *M. tuberculosis*. Two heat-shock regulons were identified. HspR acts as a transcriptional repressor for the members of the Hsp70 (DnaK) regulon, and HrcA similarly regulates the Hsp60 (GroE) response. These two specific repressor circuits overlap with broader transcriptional changes mediated by alternative sigma factors during exposure to high temperatures. Several previously undescribed heat-shock genes were identified as members of the HspR and HrcA regulons. A novel HspR-controlled operon encodes a member of the low-molecular-mass alpha-crystallin family. This protein is one of the most prominent features of the *M. tuberculosis* heat-shock response and is related to a major antigen induced in response to anaerobic stress.

L2 ANSWER 4 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 2

AN 2003:2068 BIOSIS

DN PREV200300002068

TI The heat shock response of ***Mycobacterium*** tuberculosis: Linking gene expression, immunology and pathogenesis.

AU ***Stewart, Graham R. (1)*** ; Wernisch, Lorenz; Stabler, Richard; Mangan, Joseph A.; Hinds, Jason; Laing, Ken G.; Butcher, Philip D.; Young, Douglas B.

CS (1) Department of Infectious Diseases and Microbiology, Centre for Molecular Microbiology and Infection, Imperial College of Science Technology and Medicine, London, SW7 2AZ, UK: g.stewart@ic.ac.uk UK

SO Comparative and Functional Genomics, (August 2002, 2002) Vol. 3, No. 4, pp. 348-351. print.
ISSN: 1531-6912.

DT Article

LA English

AB The regulation of heat shock protein (HSP) expression is critically important to pathogens such as ***Mycobacterium*** tuberculosis and dysregulation of the heat shock response results in increased immune recognition of the bacterium and reduced survival during chronic infection. In this study we use a whole genome spotted microarray to characterize the heat shock response of *M. tuberculosis*. We also begin a dissection of this important stress response by generating deletion mutants that lack specific transcriptional regulators and examining their transcriptional profiles under different stresses. Understanding the stimuli and mechanisms that govern heat shock in ***mycobacteria*** will allow us to relate observed in vivo expression patterns of HSPs to particular stresses and physiological conditions. The mechanisms controlling HSP expression also make attractive drug targets as part of a strategy designed to enhance immune recognition of the bacterium.

L2 ANSWER 5 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 3

AN 2002:173447 BIOSIS

DN PREV200200173447

TI Transposition of Tn4560 of *Streptomyces fradiae* in ***Mycobacterium*** smegmatis.

AU Bhatt, Apoorva (1); ***Stewart, Graham R.*** ; Kieser, Tobias

CS (1) Department of Biochemistry, University of Cambridge, 80, Tennis Court

Road, Cambridge, CB2 1GA: ab382@mole.bio.cam.ac.uk UK
SO FEMS Microbiology Letters, (10 January, 2002) Vol. 206, No. 2, pp. 241-246. print.
ISSN: 0378-1097.

DT Article
LA English
AB Tn4560 (8.6 kb) was derived from Tn4556, a Tn3-like element from *Streptomyces fradiae*. It contains a viomycin resistance gene that has not been used previously for selection in ***mycobacteria***. Tn4560, cloned in a *Streptomyces* plasmid, was introduced by electroporation into ***Mycobacterium*** *smegmatis* mc2155. Tn4560 transposed into the host genome: there was no obvious target sequence preference, and insertions were in or near several conserved open reading frames. The insertions were located far apart on different *AseI* macrorestriction fragments. Unexpectedly, the transposon delivery plasmid, pUC1169, derived from the *Streptomyces* multicopy plasmid pIJ101, replicated partially in *M. smegmatis*, but was lost spontaneously during subculture. Replication of pUC1169 probably contributed to the relatively high efficiency of Tn4560 delivery: up to 28% of the potential *M. smegmatis* transformants acquired a stable transposon insertion. The data indicated that Tn4560 may be useful for random mutagenesis of *M. smegmatis*.

L2 ANSWER 6 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 4
AN 2002:464091 BIOSIS
DN PREV200200464091
TI Tuberculosis vaccines.
AU Young, Douglas B. (1); ***Stewart, Graham R.***
CS (1) Centre for Molecular Microbiology and Infection, Imperial College of Science, Technology and Medicine, South Kensington, Flowers Building, London, SW7 2AZ UK
SO British Medical Bulletin, (2002) Vol. 62, pp. 73-86. print.
ISSN: 0007-1420.
DT General Review
LA English

L2 ANSWER 7 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 5
AN 2001:304488 BIOSIS
DN PREV200100304488
TI Overexpression of heat-shock proteins reduces survival of ***Mycobacterium*** tuberculosis in the chronic phase of infection.
AU ***Stewart, Graham R. (1)*** ; Snewin, Valerie A.; Walzl, Gerhard; Hussell, Tracy; Tormay, Peter; O'Gaora, Peadar; Goyal, Madhu; Betts, Joanna; Brown, Ivor N.; Young, Douglas B.
CS (1) Departments of Infectious Diseases and Microbiology, Centre for Molecular Microbiology and Infection, Imperial College of Science Technology and Medicine, London: g.stewart@ic.ac.uk UK
SO Nature Medicine, (June, 2001) Vol. 7, No. 6, pp. 732-737. print.
ISSN: 1078-8956.
DT Article
LA English
SL English
AB Elevated expression of heat-shock proteins (HSPs) can benefit a microbial pathogen struggling to penetrate host defenses during infection, but at the same time might provide a crucial signal alerting the host immune system to its presence. To determine which of these effects predominate,

we constructed a mutant strain of ***Mycobacterium*** tuberculosis that constitutively overexpresses Hsp70 proteins. Although the mutant was fully virulent in the initial stage of infection, it was significantly impaired in its ability to persist during the subsequent chronic phase. Induction of microbial genes encoding HSPs might provide a novel strategy to boost the immune response of individuals with latent tuberculosis infection.

L2 ANSWER 8 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 6
 AN 2001:70724 BIOSIS
 DN PREV200100070724
 TI Role of ***Mycobacterium*** tuberculosis copper-zinc superoxide
 dismutase.
 AU Dussurget, Olivier (1); ***Stewart, Graham*** ; Neyrolles, Olivier;
 Pescher, Pascale; Young, Douglas; Marchal, Gilles
 CS (1) Institut Pasteur, 28 Rue du Dr Roux, 75724, Paris Cedex 15:
 odussur@pasteur.fr France
 SO Infection and Immunity, (January, 2001) Vol. 69, No. 1, pp. 529-533.
 print.
 ISSN: 0019-9567.
 DT Article
 LA English
 SL English
 AB Superoxide dismutases (SODs) play an important role in protection against
 oxidative stress and have been shown to contribute to the pathogenicity of
 many bacterial species. To determine the function of the
 mycobacterial copper and zinc-cofactored SOD (CuZnSOD), we
 constructed and characterized ***Mycobacterium*** tuberculosis and
 Mycobacterium bovis BCG CuZnSOD null mutants. Both strains were
 more sensitive to superoxides and hydrogen peroxide than were their
 respective parental strains. The survival of M. bovis BCG in unstimulated
 as well as activated mouse bone marrow-derived macrophages was not
 affected by the loss of CuZnSOD. The survival of CuZnSOD deficient-M.
 tuberculosis in guinea pig tissues was comparable to that of its parental
 strain. These results indicate that the ***mycobacterial*** CuZnSOD is
 not essential for intracellular growth within macrophages and does not
 detectably contribute to the pathogenicity of M. tuberculosis.

L2 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 2000:903455 CAPLUS
 DN 135:221819
 TI Recombination
 AU ***Stewart, Graham R.*** ; McFadden, Johnjoe
 CS School of Biological Sciences, University of Surrey, Surrey, GU2 5XH, UK
 SO Mycobacteria (1999), 1-16. Editor(s): Ratledge, Colin; Dale, Jeremy.
 Publisher: Blackwell Science Ltd., Oxford, UK.
 CODEN: 69ATXF
 DT Conference; General Review
 LA English
 AB A review with 66 refs. on homologous recombination in both fast- and
 slow-growing ***mycobacteria***. In addn. to examg. the tech.
 difficulties involved in producing gene knockouts in ***mycobacteria***
 , it is attempted to relate exptl. observations with information from the
 M. tuberculosis genome project to produce putative biochem. pathways for
 recombination.

RE.CNT 66 THERE ARE 66 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> e ogaora peadar/au

E1 1 OGAOGA DIVI/AU
 E2 11 OGAORA P/AU
 E3 0 --> OGAORA PEADAR/AU
 E4 37 OGAR B/AU
 E5 1 OGAR BJORN/AU
 E6 1 OGAR C T/AU
 E7 8 OGAR D/AU
 E8 4 OGAR D A/AU
 E9 1 OGAR D I/AU
 E10 1 OGAR D K/AU
 E11 1 OGAR DALE/AU
 E12 1 OGAR DALE A/AU

=> s e2

L3 11 "OGAORA P"/AU

=> dup rem l3

PROCESSING COMPLETED FOR L3

L4 11 DUP REM L3 (0 DUPLICATES REMOVED)

=> d bib ab 1-

YOU HAVE REQUESTED DATA FROM 11 ANSWERS - CONTINUE? Y/(N):y

L4 ANSWER 1 OF 11 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

AN 2000:25725 SCISEARCH

GA The Genuine Article (R) Number: 270BH

TI Identification of a Mycobacterium tuberculosis gene that enhances mycobacterial survival in macrophages

AU Wei J; Dahl J L; Moulder J W; Roberts E A; ***OGaora P*** ; Young D B; Friedman R L (Reprint)

CS UNIV ARIZONA, COLL MED, DEPT MICROBIOL & IMMUNOL, 1501 N CAMPBELL AVE, TUCSON, AZ 85724 (Reprint); UNIV ARIZONA, COLL MED, DEPT MICROBIOL & IMMUNOL, TUCSON, AZ 85724; ST MARYS HOSP, IMPERIAL COLL, SCH MED, DEPT MED MICROBIOL, LONDON W2 1PG, ENGLAND

CYA USA; ENGLAND

SO JOURNAL OF BACTERIOLOGY, (JAN 2000) Vol. 182, No. 2, pp. 377-384.

Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW,

WASHINGTON, DC 20005-4171.

ISSN: 0021-9193.

DT Article; Journal

FS LIFE

LA English

REC Reference Count: 47

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Intracellular survival plays a central role in the pathogenesis of Mycobacterium tuberculosis. To identify M. tuberculosis genes required for intracellular survival within macrophages, an M. tuberculosis H37Rv plasmid library was constructed by using the shuttle vector pOLYG. This plasmid library was electroporated into Mycobacterium smegmatis 1-2c, and the transformants were used to infect the human macrophage-like cell line U-937. Because M. smegmatis does not readily survive within macrophages, any increased intracellular survival is likely due to cloned nl:

tuberculosis H37Rv DNA, After six sequential passages of nil. smegmatis transformants through U-937 cells, one clone (p69) was enriched more than 70% as determined by both restriction enzyme and PCR analyses. p69 demonstrated significantly enhanced survival compared to that of the vector control, ranging from 2.4- to 5.3-fold at both 24 and 48 h after infection. DNA sequence analysis revealed three open reading frames (ORFs) in the insert of p69. ORF2 (1.2 kb) was the only one which contained a putative promoter region and a ribosome-binding site. Deletion analysis of the p69 insert DNA showed that disruption of ORF2 resulted in complete loss of the enhanced intracellular survival phenotype. This gene was named the enhanced intracellular survival (eis) gene. By using an internal region of eis as a probe for Southern analysis, eis was found in the genomic DNA of various ill. tuberculosis strains and of Mycobacterium bovis BCG but not in that of ill. smegmatis or 10 other nonpathogenic mycobacterial species. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis showed that all M. smegmatis eis-containing constructs expressed a unique protein of 42 kDa, the predicted size of Eis. The expression of this 42-kDa protein directly correlated to the enhanced survival of nl. smegmatis p69 in U-937 cells. These results suggest a possible role for eis and its protein product in the intracellular survival of M. tuberculosis.

L4 ANSWER 2 OF 11 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
 AN 1999:224206 SCISEARCH
 GA The Genuine Article (R) Number: 175YD
 TI Construction and murine immunogenicity of recombinant Bacille Calmette
 Guerin vaccines expressing the B subunit of Escherichia coli heat labile
 enterotoxin
 AU Hayward C M M; ***OGaora P*** ; Young D B; Griffin G E; Thole J; Hirst
 T R; CastelloBranco L R R; Lewis D J M (Reprint)
 CS ST GEORGE HOSP, SCH MED, DIV INFECT DIS, LONDON SW17 0RE, ENGLAND
 (Reprint); ST GEORGE HOSP, SCH MED, DIV INFECT DIS, LONDON SW17 0RE,
 ENGLAND; UNIV LONDON IMPERIAL COLL SCI TECHNOL & MED, ST MARYS, SCH MED,
 DEPT MICROBIOL, LONDON, ENGLAND; UNIV BRISTOL, DEPT PATHOL & MICROBIOL,
 BRISTOL, AVON, ENGLAND; FDN OSWALDO CRUZ, RIO JANEIRO, BRAZIL
 CYA ENGLAND; BRAZIL
 SO VACCINE, (5 MAR 1999) Vol. 17, No. 9-10, pp. 1272-1281.
 Publisher: ELSEVIER SCI LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON,
 OXFORD OX5 1GB, OXON, ENGLAND.
 ISSN: 0264-410X.
 DT Article; Journal
 FS LIFE; AGRI
 LA English
 REC Reference Count: 27
 ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
 AB Three recombinant strains of Mycobacterium bovis Bacille Calmette
 Guerin (rBCG) were prepared in which the immunogenic B subunit of human
 Escherichia coli heat labile enterotoxin (LT-Bh) was expressed either as a
 cytoplasm protein, a cell wall associated lipoprotein or a secreted
 protein. Intraperitoneal immunisation of mice with these rBCG induced IgG
 and IgA antibodies to LT-Bh and shifted the serum IgE subclass response to
 subsequent challenge with purified LT-Bh from IgG(1) to an IgG(2a). Oral
 administration of recombinant BCG induced mucosal and serum IgA antibodies
 to LT-Bh which peaked four months after immunisation. Antibody responses
 were greater when LT-Bh was expressed as a secreted protein or lipoprotein
 rather than in the cytoplasm. Oral vaccination with recombinant BCG may be
 an effective approach, particularly to induce mucosal IgA and prime for a

serum TH1 recall response. (C) 1999 Elsevier Science Ltd. All rights reserved.

L4 ANSWER 3 OF 11 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AN 1999:103949 SCISEARCH
GA The Genuine Article (R) Number: 160VE
TI Neisseria gonorrhoeae mutants altered in toxicity to human fallopian tubes and molecular characterization of the genetic locus involved
AU Arvidson C G (Reprint); Kirkpatrick R; Witkamp M T; Larson J A; Schipper C A; Waldbeser L S; ***OGaora P*** ; Cooper M; So M
CS OREGON HLTH SCI UNIV, DEPT MOL MICROBIOL & IMMUNOL, L220, 3181 SW SAN JACKSON PK RD, PORTLAND, OR 97201 (Reprint); SO ILLINOIS UNIV, SCH MED, DEPT MED MICROBIOL & IMMUNOL, SPRINGFIELD, IL 62794
CYA USA
SO INFECTION AND IMMUNITY, (FEB 1999) Vol. 67, No. 2, pp. 643-652.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0019-9567.
DT Article; Journal
FS LIFE
LA English
REC Reference Count: 60
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB In an effort to identify potential cytotoxins expressed by Neisseria gonorrhoeae, we have identified a locus that, when mutated in the gonococcus, results in a significant increase in toxicity of the strain to human fallopian tube organ cultures (HFTOC). This locus, gly1, contains two open reading frames (ORFs) which are likely cotranscribed. ORF1 encodes a polypeptide of 17.8 kDa with a signal sequence that is recognized and processed in Escherichia coli and N. gonorrhoeae. The 15.6-kDa processed polypeptide has been observed in membrane fractions and filtered spent media from cultures of E. coli expressing gly1 and in outer membrane preparations of wild-type N. gonorrhoeae. The gly1 locus is not essential for bacterial survival, and it does not play a detectable role in epithelial cell adhesion, invasion, or intracellular survival. However, a gly1 null mutant causes much more damage to fallopian tube tissues than its isogenic wild-type parent. A strain complemented in trans for the gly1 mutation showed a level of toxicity to HFTOC similar to the level elicited by the wild-type parent. Taken together, these results indicate an involvement of the gly1 locus in the toxicity of N. gonorrhoeae to human fallopian tubes.

L4 ANSWER 4 OF 11 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AN 96:555228 SCISEARCH
GA The Genuine Article (R) Number: UY922
TI BACTERIAL GLYCOPROTEINS - A LINK BETWEEN GLYCOSYLATION AND PROTEOLYTIC CLEAVAGE OF A 19 KDA ANTIGEN FROM MYCOBACTERIUM-TUBERCULOSIS
AU HERRMANN J L (Reprint); ***OGAORA P*** ; GALLAGHER A; THOLE J E R; YOUNG D B
CS HOP ST LOUIS, MICROBIOL LAB, PARIS, FRANCE (Reprint); ST MARYS HOSP, SCH MED, IMPERIAL COLL, DEPT MED MICROBIOL, LONDON W2 1PG, ENGLAND
CYA FRANCE; ENGLAND
SO EMBO JOURNAL, (15 JUL 1996) Vol. 15, No. 14, pp. 3547-3554.
ISSN: 0261-4189.
DT Article; Journal
FS LIFE
LA ENGLISH

REC Reference Count: 54

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Protein glycosylation has an important influence on a broad range of molecular interactions in eukaryotes, but is comparatively rare in bacteria. Several antigens from Mycobacterium tuberculosis, the causative agent of human tuberculosis, have been identified as glycoproteins on the basis of lectin binding, or by detailed structural analysis. By production of a set of alkaline phosphatase (PhoA) hybrid proteins in a mycobacterial expression system, the peptide region required for glycosylation of the 19 kDa lipoprotein antigen from M. tuberculosis was defined. Mutagenesis of two threonine clusters within this region abolished lectin binding by PhoA hybrids and by the 19 kDa protein itself. Substitution of the threonine residues also resulted in generation of a series of smaller forms of the protein as a result of proteolysis. In a working model to account for these observations, we propose that the role of glycosylation is to regulate cleavage of a proteolytically sensitive linker region close to the acylated N-terminus of the protein.

L4 ANSWER 5 OF 11 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

AN 95:148762 SCISEARCH

GA The Genuine Article (R) Number: QH529

TI THE NEISSERIA-MENINGITIDIS HEMOGLOBIN RECEPTOR - ITS ROLE IN IRON UTILIZATION AND VIRULENCE

AU STOJILJKOVIC I (Reprint); HWA V; DESAINTMARTIN L; ***OGAORA P*** ; NASSIF X; HEFFRON F; SO M

CS OREGON HLTH SCI UNIV, DEPT MOLEC MICROBIOL & IMMUNOL, PORTLAND, OR, 97201 (Reprint); UNIV PARIS 05, FAC MED NECKER ENFANTS MALAD, INSERM, U411, MICROBIOL LAB, PARIS, FRANCE; ST MARYS HOSP, SCH MED, DEPT MED MICROBIOL, LONDON W2 1PG, ENGLAND

CYA USA; FRANCE; ENGLAND

SO MOLECULAR MICROBIOLOGY, (FEB 1995) Vol. 15, No. 3, pp. 531-541. ISSN: 0950-382X.

DT Article; Journal

FS LIFE

LA ENGLISH

REC Reference Count: 52

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The Neisseria meningitidis haemoglobin receptor gene, hmbR, was cloned by complementation in a porphyrin-requiring Escherichia coli mutant. hmbR encodes an 89.5kDa outer membrane protein which shares amino acid homology with the Tone-dependent receptors of Gram-negative bacteria. HmbR had the highest similarity to Neisseria transferrin and lactoferrin receptors. The utilization of haemoglobin as an iron source required internalization of the haemin moiety by the cell. The mechanism of haemin internalization via the haemoglobin receptor was TonB-dependent in E. coli. A N. meningitidis hmbR mutant was unable to use haemoglobin but could still use haemin as a sole iron source. The existence of a second N. meningitidis receptor gene, specific for haemin, was shown by the isolation of cosmids which did not hybridize with the hmbR probe, but which were able to complement an E. coli hemA aroB mutant on haemin-supplemented plates. The N. meningitidis hmbR mutant was attenuated in an infant rat model for meningococcal infection, indicating that haemoglobin utilization is important for N. meningitidis virulence.

L4 ANSWER 6 OF 11 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

AN 94:253922 SCISEARCH

GA The Genuine Article (R) Number: NJ034

TI ROLES OF PILIN AND PILC IN ADHESION OF NEISSERIA-MENINGITIDIS TO HUMAN
EPITHELIAL AND ENDOTHELIAL-CELLS

AU NASSIF X (Reprint); BERETTI J L; LOWY J; STENBERG P; ***OGAORA P*** ;
PFEIFER J; NORMARK S; SO M

CS UNIV PARIS 05, INSERM, U411, MICROBIOL LAB, 156 RUE VAUGIRARD, F-75730
PARIS 15, FRANCE (Reprint); OREGON HLTH SCI UNIV, DEPT MOLEC MICROBIOL &
IMMUNOL, PORTLAND, OR, 97201; OREGON HLTH SCI UNIV, DEPT PATHOL, PORTLAND,
OR, 97201; WASHINGTON UNIV, DEPT MOLEC MICROBIOL, ST LOUIS, MO, 63110

CYA FRANCE; USA

SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF
AMERICA, (26 APR 1994) Vol. 91, No. 9, pp. 3769-3773.
ISSN: 0027-8424.

DT Article; Journal

FS LIFE

LA ENGLISH

REC Reference Count: 21
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Pili and pilin antigenic variation play important roles in adhesion of
Neisseria meningitidis (MC) to human epithelial and endothelial cells. We
recently identified one pilin variant that confers high adhesiveness of MC
to human epithelial cells in culture. However, other factor(s) also play a
role in MC adhesiveness, since some nonadhesive variants of MC strain 8013
are piliated and produce the same pilin variant as adhesive derivatives.
PilC1 and PilC2, high molecular weight outer membrane proteins in
Neisseria gonorrhoeae, are proposed to play roles in pilus assembly.
Strain 8013 also contains pilC1 and pilC2; their products function in a
similar if not identical manner in pilus biogenesis. PilC1 has an
additional function in that it also modulates adhesiveness of strain 8013.

L4 ANSWER 7 OF 11 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

AN 93:331013 SCISEARCH

GA The Genuine Article (R) Number: LD099

TI ANTIGENIC VARIATION OF PILIN REGULATES ADHESION OF NEISSERIA-MENINGITIDIS
TO HUMAN EPITHELIAL-CELLS

AU NASSIF X (Reprint); LOWY J; STENBERG P; ***OGAORA P*** ; GANJI A; SO M

CS OREGON HLTH SCI UNIV, DEPT MICROBIOL & IMMUNOL, 3181 S W SAM JACKSON PK
RD, PORTLAND, OR, 97201 (Reprint); OREGON HLTH SCI UNIV, DEPT PATHOL,
PORTLAND, OR, 97201

CYA USA

SO MOLECULAR MICROBIOLOGY, (MAY 1993) Vol. 8, No. 4, pp. 719-725.
ISSN: 0950-382X.

DT Article; Journal

FS LIFE

LA ENGLISH

REC Reference Count: 30
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Pili have been shown to play an essential role in the adhesion of
Neisseria meningitidis to epithelial cells. However, among piliated
strains, both inter- and intrastrain variability exist with respect to
their degree of adhesion to epithelial cells in vitro (Virji et al.,
1992). This suggests that factors other than the presence of pili per se
are involved in this process. The N. meningitidis pilin subunit undergoes
extensive antigenic variation. Piliated low- and high-adhesive derivatives
of the same N. meningitidis strain were selected and the nucleotide
sequence of the pilin gene expressed in each was determined. The highly
adhesive derivatives had the same pilin sequence. The alleles encoding the
pilin subunit of the low-adhesive derivatives were completely different

from the one found in the high-adhesive isolates. Using polyclonal antibodies raised against one hyperadhesive variant, it was confirmed that the low-adhesive pilated derivatives expressed pilin variants antigenically different from the highly adhesive strains. The role of antigenic variation in the adhesive process of *N. meningitidis* was confirmed by performing allelic exchanges of the pile locus between low- and high-adhesive isolates. Antigenic variation has been considered a means by which virulent bacteria evade the host immune system. This work provides genetic proof that a bacterial pathogen, *N. meningitidis*, can use antigenic variation to modulate their degree of virulence.

L4 ANSWER 8 OF 11 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
 AN 91:133382 SCISEARCH
 GA The Genuine Article (R) Number: EZ838
 TI THE ROLE OF A STRESS-RESPONSE PROTEIN IN SALMONELLA-TYPHIMURIUM VIRULENCE
 AU JOHNSON K; CHARLES I; DOUGAN G (Reprint); PICKARD D; ***OGAORA P*** ;
 COSTA G; ALI T; MILLER I; HORMAECHE C
 CS WELLCOME BIOTECH, DEPT MOLEC BIOL, LANGLEY COURT, BECKENHAM BR3 3BS, KENT,
 ENGLAND; INST MICROBIOL, MESSINA, ITALY; UNIV CAMBRIDGE, DEPT PATHOL,
 CAMBRIDGE CB2 1QP, ENGLAND
 CYA ENGLAND; ITALY
 SO MOLECULAR MICROBIOLOGY, (1991) Vol. 5, No. 2, pp. 401-407.
 DT Article; Journal
 FS LIFE
 LA ENGLISH
 REC Reference Count: 38
 ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
 AB We recently described the use of selective transposon mutagenesis to generate a series of avirulent mutants of a pathogenic strain of *Salmonella typhimurium*. Cloning and sequencing of the insertion sites from two of these mutants reveals that both have identical locations within an open reading frame that is highly homologous to a gene, *htrA*, encoding a heat-shock protein in *Escherichia coli*. DNA sequence analysis of *S. typhimurium htrA* reveals the presence of a gene capable of encoding a protein with a calculated M(r) of 49316 that has 88.7% protein:protein homology with its *E. coli* counterpart. In *E. coli*, lesions in this gene, also known as *degP*, reduce proteolytic degradation of aberrant periplasmic proteins. Characteristics of the *S. typhimurium htrA* mutants, 046 and 014, in vivo and in vitro suggested that they are avirulent because of impaired ability to survive and/or replicate in host tissues. In vitro, the *S. typhimurium htrA* mutants 046 and 014 are not temperature-sensitive but were found to be more susceptible to oxidative stress than the parent, suggesting that they may be less able to withstand oxidative killing within macrophages.

L4 ANSWER 9 OF 11 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
 AN 90:654930 SCISEARCH
 GA The Genuine Article (R) Number: EL062
 TI YERSINIA-ENTEROCOLITICA-AROA MUTANTS AS CARRIERS OF THE B-SUBUNIT OF THE ESCHERICHIA-COLI HEAT-LABILE ENTEROTOXIN TO THE MURINE IMMUNE-SYSTEM
 AU ***OGAORA P (Reprint)*** ; ROBERTS M; BOWE F; HORMAECHE C; DEHORMAECHE R D; CAFFERKEY M; TITE J; DOUGAN G
 CS WELLCOME BIOTECH, DEPT MOLEC BIOL, BECKENHAM BR3 3BS, KENT, ENGLAND; UNIV CAMBRIDGE, DEPT PATHOL, CAMBRIDGE CB2 1QP, ENGLAND; UNIV DUBLIN TRINITY COLL, DEPT MICROBIOL, DUBLIN 2, IRELAND
 CYA ENGLAND; IRELAND
 SO MICROBIAL PATHOGENESIS, (1990) Vol. 9, No. 2, pp. 105-116.

DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 31

L4 ANSWER 10 OF 11 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AN 89:499959 SCISEARCH
GA The Genuine Article (R) Number: AQ890
TI VIRULENCE, PERSISTENCE, AND IMMUNOGENICITY OF YERSINIA-ENTEROCOLITICA O-8
AROA MUTANTS
AU BOWE F; ***OGAORA P*** ; MASKELL D; CAFFERKEY M; DOUGAN G (Reprint)
CS UNIV DUBLIN TRINITY COLL, MOYNE INST, DUBLIN 2, IRELAND
CYA IRELAND
SO INFECTION AND IMMUNITY, (1989) Vol. 57, No. 10, pp. 3234-3236.
DT Note; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 20

L4 ANSWER 11 OF 11 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AN 89:649446 SCISEARCH
GA The Genuine Article (R) Number: CE365
TI CLONING AND CHARACTERIZATION OF THE SERC AND AROA GENES OF
YERSINIA-ENTEROCOLITICA, AND CONSTRUCTION OF AN AROA MUTANT
AU ***OGAORA P*** ; MASKELL D; COLEMAN D; CAFFERKEY M; DOUGAN G (Reprint)
CS WELLCOME BIOTECH, DEPT MOLEC BIOL, LANGLEY COURT, BECKENHAM BR3 3BS, KENT,
ENGLAND; UNIV DUBLIN TRINITY COLL, MOYNE INST, DUBLIN 2, IRELAND
CYA ENGLAND; IRELAND
SO GENE, (1989) Vol. 84, No. 1, pp. 23-30.
DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 36

=> e o'gaora/au

MISMATCHED QUOTE IN EXPAND TERM
MISMATCHED QUOTE IN EXPAND TERM
MISMATCHED QUOTE IN EXPAND TERM
MISMATCHED QUOTE IN EXPAND TERM
MISMATCHED QUOTE IN EXPAND TERM
MISMATCHED QUOTE IN EXPAND TERM
MISMATCHED QUOTE IN EXPAND TERM
MISMATCHED QUOTE IN EXPAND TERM
MISMATCHED QUOTE IN EXPAND TERM

Quotation marks (or apostrophes) must be used in pairs,
one before and one after the expression you are setting
off or masking.

=> e young douglas/au

E1 2 YOUNG DOUG C/AU
E2 1 YOUNG DOUG L/AU
E3 92 --> YOUNG DOUGLAS/AU
E4 31 YOUNG DOUGLAS A/AU
E5 3 YOUNG DOUGLAS ALAN/AU
E6 1 YOUNG DOUGLAS ALEXANDER/AU
E7 148 YOUNG DOUGLAS B/AU

E8	1	YOUNG DOUGLAS BROWNLIE/AU
E9	1	YOUNG DOUGLAS CHARLES/AU
E10	2	YOUNG DOUGLAS D/AU
E11	4	YOUNG DOUGLAS E/AU
E12	49	YOUNG DOUGLAS F/AU

=> s e3-e8 and mycobacter?

L5 177 ("YOUNG DOUGLAS"/AU OR "YOUNG DOUGLAS A"/AU OR "YOUNG DOUGLAS ALAN"/AU OR "YOUNG DOUGLAS ALEXANDER"/AU OR "YOUNG DOUGLAS B"/AU .OR "YOUNG DOUGLAS BROWNLIE"/AU) AND MYCOBACTER?

=> dup rem l5

PROCESSING COMPLETED FOR L5

L6 121 DUP REM L5 (56 DUPLICATES REMOVED)

=> d bib ab 1-

YOU HAVE REQUESTED DATA FROM 121 ANSWERS - CONTINUE? Y/(N):y

L6 ANSWER 1 OF 121 USPATFULL on STN
 AN 2003:106183 USPATFULL
 TI Stress proteins and uses therefor
 IN Young, Richard A., Weston, MA, UNITED STATES
 Young, Douglas , Ruislip, UNITED KINGDOM
 PA Whitehead Institute for Biomedical Research, Cambridge, MA (U.S. corporation)
 PI US 2003073094 A1 20030417
 AI US 2002-46649 A1 20020114 (10)
 RLI Division of Ser. No. US 1994-336251, filed on 3 Nov 1994, GRANTED, Pat. No. US 6338952 Continuation-in-part of Ser. No. WO 1994-US6362, filed on 6 Jun 1994, PENDING Continuation-in-part of Ser. No. US 1993-73381, filed on 4 Jun 1993, ABANDONED Continuation-in-part of Ser. No. US 1991-804632, filed on 9 Dec 1991, ABANDONED Continuation of Ser. No. US 1989-366581, filed on 15 Jun 1989, ABANDONED Continuation-in-part of Ser. No. US 1988-207298, filed on 15 Jun 1988, ABANDONED Continuation-in-part of Ser. No. WO 1989-US2619, filed on 15 Jun 1989, UNKNOWN
 DT Utility
 FS APPLICATION
 LREP FISH & RICHARDSON PC, 225 FRANKLIN ST, BOSTON, MA, 02110
 CLMN Number of Claims: 42
 ECL Exemplary Claim: 1
 DRWN 10 Drawing Page(s)
 LN.CNT 1480

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to stress proteins and methods of modulating an individual's immune response. In particular, it relates to the use of such stress proteins in immune therapy and prophylaxis, which results in an induction or enhancement of an individual's immune response and as an immunotherapeutic agent which results in a decrease of an individual's immune response to his or her own cells. The present invention also relates to compositions comprising a stress protein joined to another component, such as a fusion protein in which a stress protein is fused to an antigen. Further, the present invention relates to a method of generating antibodies to a substance using a conjugate comprised of a stress protein joined to the substance.

L6 ANSWER 2 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 1
 AN 2003:289858 BIOSIS
 DN PREV200300289858
 TI The MPB83 antigen from ***Mycobacterium*** bovis contains O-linked
 mannose and (1 fwardw 3)-mannobiose moieties.
 AU Michell, Stephen L. (1); Whelan, Adam O.; Wheeler, Paul R.; Panico, Maria;
 Easton, Richard L.; Etienne, A. Tony; Haslam, Stuart M.; Dell, Anne;
 Morris, Howard R.; Reason, Andrew J.; Herrmann, Jean Louis; ***Young,***
 *** Douglas B.*** ; Hewinson, R. Glyn
 CS (1) DSTL, Porton Down, Salisbury, Wiltshire, SP4 0JQ, UK:
 slmichell@dstl.gov.uk UK
 SO Journal of Biological Chemistry, (May 2 2003) Vol. 278, No. 18, pp.
 16423-16432. print.
 ISSN: 0021-9258.
 DT Article
 LA English
 AB ***Mycobacterium*** tuberculosis and ***Mycobacterium*** bovis,
 the causative agents of human and bovine tuberculosis, have been reported
 to express a range of surface and secreted glycoproteins, although only
 one of these has been subjected to detailed structural analysis. We
 describe the use of a genetic system, in conjunction with lectin binding,
 to characterize the points of attachment of carbohydrate moieties to the
 polypeptide backbone of a second ***mycobacterial*** glycoprotein,
 antigen MPB83 from M. bovis. Biochemical and structural analysis of the
 native MPB83 protein and derived peptides demonstrated the presence of 3
 mannose units attached to two threonine residues. Mannose residues were
 joined by a (1 fwardw 3) linkage, in contrast to the (1 fwardw 2) linkage
 previously observed in antigen MPT32 from M. tuberculosis and the (1
 fwardw 2) and (1 fwardw 6) linkages in other ***mycobacterial***
 glycolipids and polysaccharides. The identification of glycosylated
 antigens within the M. tuberculosis complex raises the possibility that
 the carbohydrate moiety of these glycoproteins might be involved in
 pathogenesis, either by interaction with mannose receptors on host cells,
 or as targets or modulators of the cell-mediated immune response. Given
 such a possibility characterization of ***mycobacterial***
 glycoproteins is a step toward understanding their functional role and
 elucidating the mechanisms of ***mycobacterial*** glycosylation.

L6 ANSWER 3 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 2
 AN 2003:163475 BIOSIS
 DN PREV200300163475
 TI The 19-kDa ***Mycobacterium*** tuberculosis protein induces macrophage
 apoptosis through Toll-like receptor-2.
 AU Lopez, Martin; Sly, Laura M.; Luu, Yvonne; ***Young, Douglas*** ;
 Cooper, Howard; Reiner, Neil E. (1)
 CS (1) Division of Infectious Diseases, University of British Columbia, 2733
 Heather Street, Room 452D, Vancouver, BC, V5Z 3J5, Canada:
 ethan@interchange.ubc.ca Canada
 SO Journal of Immunology, (March 1 2003) Vol. 170, No. 5, pp. 2409-2416.
 print.
 ISSN: 0022-1767.
 DT Article
 LA English
 AB Macrophages infected with ***Mycobacterium*** tuberculosis undergo
 increased rates of apoptosis. Important objectives are to define the

microbial factors that cause apoptosis, the mechanisms involved and the impact on infection. The 19-kDa *M. tuberculosis* glycolipoprotein (p19) is both cell wall-associated and secreted and is a candidate virulence factor. We investigated the potential of recombinant, His-tagged p19 lacking the secretion/acylation signal to induce macrophage apoptosis. The TUNEL assay and annexin V binding to membrane phosphatidylserine were used to measure apoptosis. The results show that p19 does act to induce apoptosis in differentiated THP-1 cells and monocyte-derived macrophages and that this effect is both dose- and time-dependent. Furthermore, this effect of p19 is Toll-like receptor (TLR)-2-mediated because preincubation of either THP-1 cells or TLR-2-expressing CHO cells with anti-TLR-2 mAb inhibited apoptosis induced by p19. Apoptosis of macrophages in response to p19 was found to be caspase-8 dependent and caspase-9 independent consistent with a transmembrane pathway signaling cell death through TLR-2. The viability of *M. tuberculosis* in cells undergoing apoptosis induced by p19 was significantly reduced suggesting the possibility that this may favor containment of infection. Although native p19 is a

mycobacterial glycolipoprotein, based upon the use of recombinant p19 where the acylation signal had been removed, we conclude that it is the polypeptide component of p19 that is responsible for signaling through TLR-2 and that the lipid moiety is not required.

L6 ANSWER 4 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 3
 AN 2003:339338 BIOSIS
 DN PREV200300339338
 TI Failure to control growth of ***mycobacteria*** in blood from children infected with human immunodeficiency virus and its relationship to T cell function.
 AU Tena, Gwen N.; ***Young, Douglas B.*** ; Eley, Brian; Henderson, Howard; Nicol, Mark P.; Levin, Mike; Kampmann, Beate (1)
 CS (1) Dept. of Paediatrics, Imperial College of Medicine, Norfolk Place, St. Mary's Campus, London, W2 1NY, UK: b.kampmann@ic.ac.uk UK
 SO Journal of Infectious Diseases, (15 May 2003) Vol. 187, No. 10, pp. 1544-1551. print.
 ISSN: 0022-1899.
 DT Article
 LA English
 AB The mechanisms of protective immunity to tuberculosis remain poorly understood in humans. A whole-blood infection model that employs a luminescent readout was used to analyze the role of T cells in control of ***mycobacterial*** infection. Control of ***mycobacterial*** growth in blood from healthy tuberculin-positive individuals was shown to be mediated predominantly by CD4+ T cells. Comparison of age-matched cohorts of human immunodeficiency virus (HIV)-infected and -uninfected children from South Africa demonstrated an association between low CD4 cell counts, low interferon (IFN)-gamma production, and impaired ability to regulate growth of ***Mycobacterium*** bovis bacille Calmette-Guerin in blood from HIV-infected children. Impaired control of infection was not reconstituted by the addition of exogenous IFN-gamma. The whole-blood assay provides an important tool for monitoring and dissecting of human immune responses to ***mycobacterial*** infection.

L6 ANSWER 5 OF 121 MEDLINE on STN
 AN 2003432660 IN-PROCESS
 DN 22854253 PubMed ID: 12972340

TI Conquistadors and ***Mycobacterium*** bovis.
 AU ***Young Douglas***
 CS Centre for Molecular Microbiology and Infection, Flowers Building,
 Imperial College, SW7 2AZ, London, UK.
 SO Tuberculosis (Edinb), (2003) 83 (5) 277-8.
 Journal code: 100971555. ISSN: 1472-9792.
 CY England: United Kingdom
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS IN-PROCESS; NONINDEXED; Priority Journals
 ED Entered STN: 20030916
 Last Updated on STN: 20030916

L6 ANSWER 6 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 4
 AN 2003:355012 BIOSIS
 DN PREV200300355012
 TI Ten years of research progress and what's to come.
 AU ***Young, Douglas B. (1)***
 CS (1) Centre for Molecular Microbiology and Infection, Faculty of Medicine,
 Imperial College London, Flowers Building, London, SW7 2AZ, UK:
 d.young@ic.ac.uk UK
 SO Tuberculosis (Amsterdam), (2003) Vol. 83, No. 1, pp. 77-81. print.
 ISSN: 1472-9792.
 DT General Review
 LA English
 AB There has been a renaissance in interest in tuberculosis research over the
 last decade. A search of the National Library of Medicine database records
 an output of 246 papers on ***Mycobacterium*** tuberculosis in 1980.
 This had risen to 615 in 1990, to over 1000 in 1995, and to 1537 in the
 year 2000. This increase has been stimulated by heightened awareness
 amongst the research community of the magnitude of the global burden of
 tuberculosis, by increased funding, and by new scientific opportunities
 provided by advances in genomics and in cellular immunology.

L6 ANSWER 7 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 5
 AN 2003:234267 BIOSIS
 DN PREV200300234267
 TI Prospects for molecular epidemiology of leprosy.
 AU ***Young, Douglas (1)***
 CS (1) CMMI, Imperial College London, Flowers Building, London, UK:
 d.young@imperial.ac.uk UK
 SO Leprosy Review, (March 2003, 2003) Vol. 74, No. 1, pp. 11-17. print.
 ISSN: 0305-7518.
 DT Article
 LA English

L6 ANSWER 8 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 2002:675871 CAPLUS
 DN 137:200259
 TI Vaccine compositions comprising modified pathogen overexpressing heat
 shock protein for therapeutic intervention in infectious disease
 IN ***Young, Douglas Brownlie*** ; Stewart, Graham Roger; O'Gaora, Peadar
 Caoimhin Eoin
 PA Sequella, Inc., USA
 SO PCT Int. Appl., 59 pp.

CODEN: PIXXD2

DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	-----	---	-----	-----	-----
PI	WO 2002067982	A2	20020906	WO 2002-US5038	20020220
	W:				
	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,				
	CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,				
	GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,				
	LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,				
	PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,				
	UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU,				
	TJ, TM				
	RW:				
	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,				
	CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,				
	BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	US 2002172685	A1	20021121	US 2002-79136	20020220
PRAI	US 2001-269801P	P	20010220		
	US 2001-294170P	P	20010529		

AB Methods and compns. for the treatment and prevention of infectious diseases are provided. In particular, efficient vaccines comprising genetically modified pathogens are provided. The vaccines generally comprise ***mycobacterial*** mutants having modified protein prodn. capabilities. In one embodiment, the mutants overexpress heat shock protein. In a specific embodiment, the ***mycobacterial*** mutant overexpresses heat shock proteins 60 and/or 70. Also provided are modified BCG vaccines capable of overexpressing heat shock proteins 60 and/or 70.

L6 ANSWER 9 OF 121 USPATFULL on STN

AN 2002:307566 USPATFULL

TI Methods and compositions for therapeutic intervention in infectious disease

IN Stewart, Graham, Walton-on-Thames, UNITED KINGDOM

O'Gaora, Peadar, London, UNITED KINGDOM

Young, Douglas, Ruislip, UNITED KINGDOM

PI US 2002172685 A1 20021121

AI US 2002-79136 A1 20020220 (10)

PRAI US 2001-269801P 20010220 (60)

US 2001-294170P 20010529 (60)

DT Utility

FS APPLICATION

LREP JOHN S. PRATT, ESQ, KILPATRICK STOCKTON, LLP, 1100 PEACHTREE STREET, SUITE 2800, ATLANTA, GA, 30309

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 15 Drawing Page(s)

LN.CNT 1922

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods and compositions for the treatment and prevention of infectious diseases are provided. In particular, efficient vaccines comprising genetically modified pathogens are provided. The vaccines generally comprise ***mycobacterial*** mutants having modified protein production capabilities. In one embodiment, the mutants overexpress heat shock protein. In a specific embodiment, the ***mycobacterial*** mutant overexpresses heat shock proteins 60 and/or 70. Also provided are

modified BCG vaccines capable of overexpressing heat shock proteins 60 and/or 70.

L6 ANSWER 10 OF 121 USPATFULL on STN
AN 2002:1089 USPATFULL
TI Stress proteins and uses therefor
IN Young, Richard A., Weston, MA, United States
Young, Douglas, Ruislip, UNITED KINGDOM
PA Whitehead Institute for Biomedical Research, Cambridge, MA, United States (U.S. corporation)
PI US 6335183 B1 20020101
AI US 1995-461722 19950605 (8)
RLI Continuation of Ser. No. US 1994-336251, filed on 3 Nov 1994, now abandoned Continuation-in-part of Ser. No. WO 1994-US6362, filed on 6 Jun 1994 Continuation-in-part of Ser. No. US 1993-73381, filed on 4 Jun 1993, now abandoned Continuation-in-part of Ser. No. US 1991-804632, filed on 9 Dec 1991, now abandoned Continuation of Ser. No. US 1989-366581, filed on 15 Jun 1989, now abandoned Continuation-in-part of Ser. No. WO 1989-US2619, filed on 15 Jun 1989 Continuation-in-part of Ser. No. US 1988-207298, filed on 15 Jun 1988, now abandoned
DT Utility
FS GRANTED
EXNAM Primary Examiner: Park, Hankyel T.; Assistant Examiner: Brown, Stacy S.
LREP Hamilton, Brook, Smith & Reynolds, P.C.
CLMN Number of Claims: 36
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 10 Drawing Page(s)
LN.CNT 1508
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to stress proteins and methods of modulating an individual's immune response. In particular, it relates to the use of such stress proteins in immune therapy and prophylaxis, which results in an induction or enhancement of an individual's immune response and as an immunotherapeutic agent which results in a decrease of an individual's immune response to his or her own cells. The present invention also relates to compositions comprising a stress protein joined to another component, such as a fusion protein in which a stress protein is fused to an antigen. Further, the present invention relates to a method of generating antibodies to a substance using a conjugate comprised of a stress protein joined to the substance.

L6 ANSWER 11 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2002:234036 BIOSIS
DN PREV200200234036
TI Immune intervention in tuberculosis.
AU ***Young, Douglas B. (1)***; Robertson, Brian D. (1)
CS (1) Department of Infectious Diseases and Microbiology, Faculty of Medicine, Imperial College, London, W2 1PG UK
SO Kaufmann, Stefan H. E. [Editor]; Sher, Alan [Editor]; Ahmed, Rafi [Editor]. (2002) pp. 439-451. Immunology of infectious diseases. Edition. 1. print.
Publisher: ASM Press 1752 N St. NW, Washington, DC, 20036-2904, USA.
ISBN: 1-55581-214-7 (cloth).
DT Book
LA English

L6 ANSWER 12 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

DUPLICATE 6

AN 2002:600499 BIOSIS
DN PREV200200600499
TI Dissection of the heat-shock response in ***Mycobacterium***
tuberculosis using mutants and microarrays.
AU Stewart, Graham R. (1); Wernisch, Lorenz; Stabler, Richard; Mangan, Joseph
A.; Hinds, Jason; Laing, Ken G.; ***Young, Douglas B.*** ; Butcher,
Philip D.
CS (1) Department of Infectious Diseases and Microbiology, Centre for
Molecular Microbiology and Infection, Imperial College of Science
Technology and Medicine, London, SW7 2AZ: g.stewart@ic.ac.uk UK
SO Microbiology (Reading), (October, 2002) Vol. 148, No. 10, pp. 3129-3138.
<http://mic.sgmjournals.org>. print.
ISSN: 1350-0872.
DT Article
LA English
AB Regulation of the expression of heat-shock proteins plays an important
role in the pathogenesis of ***Mycobacterium*** tuberculosis. The
heat-shock response of bacteria involves genome-wide changes in gene
expression. A combination of targeted mutagenesis and whole-genome
expression profiling was used to characterize transcription factors
responsible for control of genes encoding the major heat-shock proteins of
M. tuberculosis. Two heat-shock regulons were identified. HspR acts as a
transcriptional repressor for the members of the Hsp70 (DnaK) regulon, and
HrcA similarly regulates the Hsp60 (GroE) response. These two specific
repressor circuits overlap with broader transcriptional changes mediated
by alternative sigma factors during exposure to high temperatures. Several
previously undescribed heat-shock genes were identified as members of the
HspR and HrcA regulons. A novel HspR-controlled operon encodes a member of
the low-molecular-mass alpha-crystallin family. This protein is one of the
most prominent features of the M. tuberculosis heat-shock response and is
related to a major antigen induced in response to anaerobic stress.

L6 ANSWER 13 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 7

AN 2002:600470 BIOSIS
DN PREV200200600470
TI An ex vivo culture model for screening drug activity against in vivo
phenotypes of ***Mycobacterium*** tuberculosis.
AU Turner, David J.; Hoyle, Stefan L.; Snewin, Valerie A.; Gares,
Marie-Pierre; Brown, Ivor N.; ***Young, Douglas B. (1)***
CS (1) Centre for Molecular Microbiology and Infection, Department of
Infectious Diseases and Microbiology, Faculty of Medicine, Imperial
College of Science, Technology and Medicine, London, SW7 2AZ:
d.young@ic.ac.uk UK
SO Microbiology (Reading), (October, 2002) Vol. 148, No. 10, pp. 2929-2936.
<http://mic.sgmjournals.org>. print.
ISSN: 1350-0872.
DT Article
LA English
AB Since the activity of drugs against ***Mycobacterium*** tuberculosis
grown in microbiological culture can differ from their activity against
bacteria present in infected tissues; compounds with optimal activity
against in vivo phenotypes may be overlooked in drug-discovery programmes
that rely on in vitro screens. The authors have investigated the use of an
ex vivo cell-culture model to assess the action of drugs on M.
tuberculosis in an environment resembling that encountered during

infection. ***Mycobacterial*** viability in the ex vivo model was shown to be regulated by the cell-mediated immune system, with growth inhibited by CD4+ T cells at an early stage of infection in BCG-vaccinated mice, and at a later stage after infection in naive mice. Screening of drugs in the ex vivo model demonstrated a window of pyrazinamide susceptibility that coincides with the onset of the T-cell-mediated immune response in naive or vaccinated mice. It is proposed that pyrazinamide acts on a population of bacteria that are exposed to an acidic environment as a result of immune activation. Clinically, administration of pyrazinamide during the initial phase of treatment reduces the risk of relapse after 6 months, suggesting that the early pyrazinamide-susceptible population may contribute to the later pool of ***mycobacteria*** that persist during prolonged chemotherapy.

L6 ANSWER 14 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 8
 AN 2002:600465 BIOSIS
 DN PREV200200600465
 TI ***Mycobacteria*** research in the post-genomic era.
 AU ***Young, Douglas B. (1)***
 CS (1) Centre for Molecular Microbiology and Infection, Department of
 Infectious Diseases and Microbiology, Faculty of Medicine, Imperial
 College of Science, Technology and Medicine, London, SW7 2AZ:
 d.young@ic.ac.uk UK
 SO Microbiology (Reading), (October, 2002) Vol. 148, No. 10, pp. 2915-2917.
 http://mic.sgmjournals.org. print.
 ISSN: 1350-0872.
 DT Article
 LA English

L6 ANSWER 15 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 9
 AN 2002:580910 CAPLUS
 DN 137:275565
 TI Spoligotyping of ***Mycobacterium*** tuberculosis isolates from
 multiple-drug-resistant tuberculosis patients from Bombay, India
 AU Mistry, Nerges F.; Iyer, Anand M.; D'souza, Desiree T. B.; Taylor, G.
 Michael; ***Young, Douglas B.*** ; Antia, Noshir H.
 CS The Foundation for Medical Research, Bombay, 400018, India
 SO Journal of Clinical Microbiology (2002), 40(7), 2677-2680
 CODEN: JCMIDW; ISSN: 0095-1137
 PB American Society for Microbiology
 DT Journal
 LA English
 AB Spoligotyping was undertaken in 65 multiple-drug-resistant
 Mycobacterium tuberculosis isolates from Bombay, India. The
 spoligotype patterns showed seven closely related clusters, a cluster with
 2 Beijing-like isolates, and unique spoligotypes (43%). Of the clusters,
 one with 29% of all the isolates suggested transmission of a dominant
 resistant clone.

RE.CNT 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 16 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 10
 AN 2003:31676 BIOSIS
 DN PREV200300031676
 TI Chronic bacterial infections: Living with unwanted guests.

AU ***Young, Douglas*** ; Hussell, Tracy; Dougan, Gordon (1)
CS (1) Centre for Molecular Microbiology and Infection, Imperial College of
Science, Technology and Medicine, London, SW7 2AZ, UK: g.dougan@ic.ac.uk
UK
SO Nature Immunology, (November 2002, 2002) Vol. 3, No. 11, pp. 1026-1032.
print.
ISSN: 1529-2908.
DT General Review
LA English
AB Some bacterial pathogens can establish life-long chronic infections in
their hosts. Persistence is normally established after an acute infection
period involving activation of both the innate and acquired immune
systems. Bacteria have evolved specific pathogenic mechanisms and harbor
sets of genes that contribute to the establishment of a persistent
lifestyle that leads to chronic infection. Persistent bacterial infection
may involve occupation of a particular tissue type or organ or
modification of the intracellular environment within eukaryotic cells.
Bacteria appear to adapt their immediate environment to favor survival and
may hijack essential immunoregulatory mechanisms designed to minimize
immune pathology or the inappropriate activation of immune effectors.

L6 ANSWER 17 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:46591 CAPLUS
DN 137:31663
TI Immune intervention in tuberculosis
AU ***Young, Douglas B.*** ; Robertson, Brian D.
CS Department of Infectious Diseases and Microbiology, Imperial College,
Faculty of Medicine, London, W2 1PG, UK
SO Immunology of Infectious Diseases (2002), 439-451. Editor(s): Kaufmann,
Stefan H. E.; Sher, Alan; Ahmed, Rafi. Publisher: American Society for
Microbiology, Washington, D. C.
CODEN: 69CEM3; ISBN: 1-55581-214-7
DT Conference; General Review
LA English
AB A review discussing the prospects for tuberculosis control by developing
improved immune interventions. It focuses on the notion that in spite of
the success of the classical vaccine paradigm in other diseases,
consideration of immune interventions in tuberculosis should be broadened
beyond the concept of mimicking the natural infection in advance encounter
with the pathogen. A brief summary of the history of immune intervention
in tuberculosis is provided.

RE.CNT 96 THERE ARE 96 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 18 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 11
AN 2002:462276 BIOSIS
DN PREV200200462276
TI Characterization of ***mycobacterial*** protein glycosyltransferase
activity using synthetic peptide acceptors in a cell-free assay.
AU Cooper, Howard N.; Gurçha, Sudagar S.; Nigou, Jerome; Brennan, Patrick J.;
Belisle, John T.; Besra, Gurdyal S.; ***Young, Douglas (1)***
CS (1) Centre for Molecular Microbiology and Infection, Imperial College of
Science, Technology and Medicine, South Kensington, London, SW7 2AZ:
d.young@ic.ac.uk UK
SO Glycobiology, (July, 2002) Vol. 12, No. 7, pp. 427-434. print.
ISSN: 0959-6658.

DT Article
 LA English
 AB Synthetic peptides derived from a 45-kDa glycoprotein antigen of ***Mycobacterium*** tuberculosis were shown to function as glycosyltransferase acceptors for mannose residues in a mannosyltransferase cell-free assay. The mannosyltransferase activity was localized within both isolated membranes and a P60 cell wall fraction prepared from the rapidly growing ***mycobacterial*** strain, ***Mycobacterium*** smegmatis. Incorporation of radiolabel from GDP-(14C)mannose was inhibited by the addition of amphomycin, indicating that the glycosyl donor for the peptide acceptors was a member of the ***mycobacterial*** polyprenol-P-mannose (PPM) family of activated glycosyl donors. Furthermore, a direct demonstration of transfer from the in situ generated PP(14C)Ms was also demonstrated. It was also found that the enzyme activity was sensitive to changes in overall peptide length and amino acid composition. Because glycoproteins are present on the ***mycobacterial*** cell surface and are available for interaction with host cells during infection, protein glycosyltransferases may provide novel drug targets. The development of a cell-free mannosyltransferase assay will now facilitate the cloning and biochemical characterisation of the relevant enzymes from M. tuberculosis.

L6 ANSWER 19 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 12

AN 2003:2068 BIOSIS

DN PREV200300002068

TI The heat shock response of ***Mycobacterium*** tuberculosis: Linking gene expression, immunology and pathogenesis.

AU Stewart, Graham R. (1); Wernisch, Lorenz; Stabler, Richard; Mangan, Joseph A.; Hinds, Jason; Laing, Ken G.; Butcher, Philip D.; ***Young, Douglas***
 *** B.***

CS (1) Department of Infectious Diseases and Microbiology, Centre for Molecular Microbiology and Infection, Imperial College of Science Technology and Medicine, London, SW7 2AZ, UK: g.stewart@ic.ac.uk UK

SO Comparative and Functional Genomics, (August 2002, 2002) Vol. 3, No. 4, pp. 348-351. print.
 ISSN: 1531-6912.

DT Article

LA English

AB The regulation of heat shock protein (HSP) expression is critically important to pathogens such as ***Mycobacterium*** tuberculosis and dysregulation of the heat shock response results in increased immune recognition of the bacterium and reduced survival during chronic infection. In this study we use a whole genome spotted microarray to characterize the heat shock response of M. tuberculosis. We also begin a dissection of this important stress response by generating deletion mutants that lack specific transcriptional regulators and examining their transcriptional profiles under different stresses. Understanding the stimuli and mechanisms that govern heat shock in ***mycobacteria*** will allow us to relate observed in vivo expression patterns of HSPs to particular stresses and physiological conditions. The mechanisms controlling HSP expression also make attractive drug targets as part of a strategy designed to enhance immune recognition of the bacterium.

L6 ANSWER 20 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 13

AN 2002:464091 BIOSIS

DN PREV200200464091
 TI Tuberculosis vaccines.
 AU ***Young, Douglas B. (1)*** ; Stewart, Graham R.
 CS (1) Centre for Molecular Microbiology and Infection, Imperial College of
 Science, Technology and Medicine, South Kensington, Flowers Building,
 London, SW7 2AZ UK
 SO British Medical Bulletin, (2002) Vol. 62, pp. 73-86. print.
 ISSN: 0007-1420.
 DT General Review
 LA English

L6 ANSWER 21 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 14
 AN 2002:140443 BIOSIS
 DN PREV200200140443
 TI Engineering of an intersubunit disulfide bridge in the iron-superoxide
 dismutase of ***Mycobacterium*** tuberculosis.
 AU Bunting, Karen A. (1); Cooper, Jonathan B.; Tickle, Ian J.; ***Young,***
 *** Douglas B.***
 CS (1) Section of Structural Biology, Institute of Cancer Research, 237
 Fulham Road, London, SW3 6JB: kbunting@icr.ac.uk UK
 SO Archives of Biochemistry and Biophysics, (January 1, 2002) Vol. 397, No.
 1, pp. 69-76. print.
 ISSN: 0003-9861.
 DT Article
 LA English
 AB With the aim of enhancing interactions involved in dimer formation, an
 intersubunit disulfide bridge was engineered in the superoxide dismutase
 enzyme of ***Mycobacterium*** tuberculosis. Ser-123 was chosen for
 mutation to cysteine since it resides at the dimer interface where the
 serine side chain interacts with the same residue in the opposite subunit.
 Gel electrophoresis and X-ray crystallographic studies of the expressed
 mutant confirmed formation of the disulfide bond under nonreducing
 conditions. However, the mutant protein was found to be less stable than
 the wild type as judged by susceptibility to denaturation in the presence
 of guanidine hydrochloride. Decreased stability probably results from
 formation of a disulfide bridge with a suboptimal torsion angle and
 exclusion of solvent molecules from the dimer interface.

L6 ANSWER 22 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 15
 AN 2001:541261 BIOSIS
 DN PREV200100541261
 TI Enhanced antimycobacterial response to recombinant ***Mycobacterium***
 bovis BCG expressing latency-associated peptide.
 AU Marshall, Ben G. (1); Wangoo, Arun; O'Gaora, Peadar; Cook, H. Terry; Shaw,
 Rory J.; ***Young, Douglas B.***
 CS (1) Department of Respiratory Medicine, Southampton University Hospitals
 NHS Trust, Southampton, Hampshire: ben.marshall@suht.swest.nhs.uk UK
 SO Infection and Immunity, (November, 2001) Vol. 69, No. 11, pp. 6676-6682.
 print.
 ISSN: 0019-9567.
 DT Article
 LA English
 SL English
 AB With a view to exploring the role of transforming growth factor beta
 (TGF-beta) during ***mycobacterial*** infection, recombinant clones of

bacillus Calmette-Guerin (BCG) were engineered to express the natural antagonist of TGF-beta, latency-activated peptide (LAP). Induction of TGF-beta activity was reduced when macrophages were infected with BCG expressing the LAP construct (LAP-BCG). There was a significant reduction in the growth of LAP-BCG in comparison to that of control BCG following intravenous infection in a mouse model. The enhanced control of

mycobacterial replication was associated with an increase in the production of gamma interferon by splenocytes challenged during the acute stage of infection but with a diminished recall response assessed after 13 weeks. Organ weight and hydroxyproline content, representing tissue pathology, were also lower in mice infected with LAP-BCG. The results are consistent with the hypothesis that TGF-beta has a detrimental effect on

mycobacterial immunity. While a reduction in TGF-beta activity augments the initial response to BCG vaccination, early bacterial clearance may adversely affect the induction of a long-term memory response by LAP-BCG.

L6 ANSWER 23 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2001:167113 CAPLUS

DN 134:325161

TI Contribution of Th1 and Th2 cells to protection and pathology in experimental models of granulomatous lung disease

AU Wangoo, Arun; Sparer, Tim; Brown, Ivor N.; Snewin, Valerie A.; Janssen, Riny; Thole, Jelle; Cook, H. Terence; Shaw, Rory J.; ***Young, Douglas***

*** B.***

CS Department of Respiratory Medicine, National Heart and Lung Institute, Imperial College School of Medicine, London, W2 1PG, UK

SO Journal of Immunology (2001), 166(5), 3432-3439

CODEN: JOIMA3; ISSN: 0022-1767

PB American Association of Immunologists

DT Journal

LA English

AB Mice that had received adoptive transfer of DO11.10 TCR transgenic T cells polarized toward a Th1 or a Th2 phenotype were challenged with Ag-coated beads or with recombinant ***Mycobacterium*** tuberculosis expressing the OVA determinant. The resulting bead-induced pulmonary granulomas reflected the phenotype of the adoptively transferred T cells, with the Th2 cells promoting a fibrotic reaction. Mice receiving Th1 cells mounted an epitope-specific protective response to challenge with recombinant M. tuberculosis. Th2 recipients were characterized by enhanced wt. loss and lung fibrosis during acute high-dose infection. The combination of TCR transgenic T cells and epitope-tagged ***mycobacteria*** provides a novel exptl. model for investigation of the pathogenesis of tuberculosis.

RE.CNT 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 24 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 16

AN 2001:185941 BIOSIS

DN PREV200100185941

TI ***Mycobacterium*** tuberculosis 19-kilodalton lipoprotein inhibits
Mycobacterium smegmatis-induced cytokine production by human
macrophages in vitro.

AU Post, Frank A.; Manca, Claudia; Neyrolles, Olivier; Ryffel, Bernhard;
Young, Douglas B. ; Kaplan, Gilla (1)

CS (1) Laboratory of Cellular Physiology and Immunology, The Rockefeller
University, 1230 York Ave., New York, NY, 10021:

kaplang@rockvax.rockefeller.edu USA

SO Infection and Immunity, (March, 2001) Vol. 69, No. 3, pp. 1433-1439.
print.
ISSN: 0019-9567.

DT Article

LA English

SL English

AB Vaccination of mice with ***Mycobacterium*** vaccae or M. smegmatis induces some protection against M. tuberculosis challenge. The 19-kDa lipoprotein of M. tuberculosis, expressed in M. vaccae or M. smegmatis (M. smeg19kDa), abrogates this protective immunity. To investigate the mechanism of this suppression of immunity, human monocyte-derived macrophages (MDM) were infected with M. smeg19kDa. Infection resulted in reduced production of tumor necrosis factor alpha (TNF-alpha) ($P < 0.01$), interleukin-12 (IL-12) ($P < 0.05$), IL-6 ($P < 0.05$), and IL-10 ($P < 0.05$), compared to infection with M. smegmatis vector (M. smegV). Infection with M. smeg19kDa and with M. smegV had no differential effect on expression of costimulatory molecules on MDM, nor did it affect the proliferation of presensitized T cells cocultured with infected MDM. When MDM were infected with M. smegmatis expressing mutated forms of the 19-kDa lipoprotein, including non-O-glycosylated (M. smeg19NOG), nonsecreted (M. smeg19NS), and nonacylated (M. smeg19NA) variants, the reduced production of TNF-alpha or IL-12 was not observed. When the purified 19-kDa lipoprotein was added directly to cultures of infected monocytes, there was little effect on either induction of cytokine production or its inhibition. Thus, the immunosuppressive effect is dependent on glycosylated and acylated 19-kDa lipoprotein present in the phagosome containing the ***mycobacterium***. These results suggest that the diminished protection against challenge with M. tuberculosis seen in mice vaccinated with M. smegmatis expressing the 19-kDa lipoprotein is the result of reduced TNF-alpha and IL-12 production, possibly leading to reduced induction of T-cell activation.

L6 ANSWER 25 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2001:925162 CAPLUS

DN 136:197937

TI Coronin is involved in uptake of ***Mycobacterium*** bovis BCG in human macrophages but not in phagosome maintenance

AU Schuller, Stephanie; Neefjes, Jacques; Ottenhoff, Tom; Thole, Jelle; ***Young, Douglas***

CS Department of Infectious Diseases and Microbiology, Imperial College School of Medicine, London, UK

SO Cellular Microbiology (2001), 3(12), 785-793
CODEN: CEMIF5; ISSN: 1462-5814

PB Blackwell Science Ltd.

DT Journal

LA English

AB By applying d. gradient electrophoresis (DGE) to human macrophages infected with ***Mycobacterium*** bovis BCG, we were able to sep. three different bacterial fractions representing arrested phagosomes, phagolysosomes and ***mycobacterial*** clumps. After further purifn. of the phagosomal population, we found that isolated phagosomes contg. live BCG were arrested in maturation as they exhibited only low amts. of the lysosomal glycoprotein LAMP-1 and processing of the lysosomal hydrolase cathepsin D was blocked. In addn., low amts. of MHC class I and class II mols. and the absence of HLA-DM suggest sequestration of ***mycobacterial*** phagosomes from antigen-processing pathways. We

further investigated the involvement of the actin-binding protein coronin in intracellular survival of ***mycobacteria*** and showed that human coronin, as well as F-actin, were assocd. with early stages of ***mycobacterial*** phagocytosis but not with phagosome maintenance. Therefore, we conclude that the unique DGE migration pattern of arrested phagosomes is not as a result of retention of coronin, but that there are other proteins or lipids responsible for the block in maturation in human macrophages.

RE.CNT 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

- L6 ANSWER 26 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 17
AN 2001:304488 BIOSIS
DN PREV200100304488
TI Overexpression of heat-shock proteins reduces survival of
 Mycobacterium tuberculosis in the chronic phase of infection.
AU Stewart, Graham R. (1); Snewin, Valerie A.; Walzl, Gerhard; Hussell,
 Tracy; Tormay, Peter; O'Gaora, Peadar; Goyal, Madhu; Betts, Joanna; Brown,
 Ivor N.; ***Young, Douglas B.***
CS (1) Departments of Infectious Diseases and Microbiology, Centre for
 Molecular Microbiology and Infection, Imperial College of Science
 Technology and Medicine, London: g.stewart@ic.ac.uk UK
SO Nature Medicine, (June, 2001) Vol. 7, No. 6, pp. 732-737. print.
 ISSN: 1078-8956.
DT Article
LA English
SL English
AB Elevated expression of heat-shock proteins (HSPs) can benefit a microbial
 pathogen struggling to penetrate host defenses during infection, but at
 the same time might provide a crucial signal alerting the host immune
 system to its presence. To determine which of these effects predominate,
 we constructed a mutant strain of ***Mycobacterium*** tuberculosis
 that constitutively overexpresses Hsp70 proteins. Although the mutant was
 fully virulent in the initial stage of infection, it was significantly
 impaired in its ability to persist during the subsequent chronic phase.
 Induction of microbial genes encoding HSPs might provide a novel strategy
 to boost the immune response of individuals with latent tuberculosis
 infection.
- L6 ANSWER 27 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 18
AN 2001:70724 BIOSIS
DN PREV200100070724
TI Role of ***Mycobacterium*** tuberculosis copper-zinc superoxide
 dismutase.
AU Dussurget, Olivier (1); Stewart, Graham; Neyrolles, Olivier; Pescher,
 Pascale; ***Young, Douglas*** ; Marchal, Gilles
CS (1) Institut Pasteur, 28 Rue du Dr Roux, 75724, Paris Cedex 15:
 odussur@pasteur.fr France
SO Infection and Immunity, (January, 2001) Vol. 69, No. 1, pp. 529-533.
 print.
 ISSN: 0019-9567.
DT Article
LA English
SL English
AB Superoxide dismutases (SODs) play an important role in protection against

oxidative stress and have been shown to contribute to the pathogenicity of many bacterial species. To determine the function of the

mycobacterial copper and zinc-cofactored SOD (CuZnSOD), we constructed and characterized ***Mycobacterium*** tuberculosis and ***Mycobacterium*** bovis BCG CuZnSOD null mutants. Both strains were more sensitive to superoxides and hydrogen peroxide than were their respective parental strains. The survival of M. bovis BCG in unstimulated as well as activated mouse bone marrow-derived macrophages was not affected by the loss of CuZnSOD. The survival of CuZnSOD deficient-M. tuberculosis in guinea pig tissues was comparable to that of its parental strain. These results indicate that the ***mycobacterial*** CuZnSOD is not essential for intracellular growth within macrophages and does not detectably contribute to the pathogenicity of M. tuberculosis.

L6 ANSWER 28 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2001:26332 CAPLUS

DN 134:206475

TI Lipoprotein access to MHC class I presentation during infection of murine macrophages with live ***mycobacteria***

AU Neyrolles, Olivier; Gould, Keith; Gares, Marie-Pierre; Brett, Sara; Janssen, Riny; O'Gaora, Peadar; Herrmann, Jean-Louis; Prevost, Marie-Christine; Perret, Emmanuelle; Thole, Jelle E. R.; ***Young,***
*** Douglas***

CS Department of Infectious Diseases and Microbiology, Imperial College School of Medicine, London, UK

SO Journal of Immunology (2001), 166(1), 447-457

CODEN: JOIMA3; ISSN: 0022-1767

PB American Association of Immunologists

DT Journal

LA English

AB Following uptake by macrophages, live ***mycobacteria*** initially reside within an immature phagosome that resists acidification and retains access to recycling endosomes. Glycolipids are exported from the ***mycobacterial*** phagosome and become available for immune recognition by CD1-restricted T cells. The aim of this study was to explore the possibility that lipoproteins might similarly escape from the phagosome and act as immune targets in cells infected with live ***mycobacteria***. We have focused on a 19-kDa lipoprotein from ***Mycobacterium*** tuberculosis that was previously shown to be recognized by CD8+ T cells. The 19-kDa Ag was found to traffic sep. from live ***mycobacteria*** within infected macrophages by a pathway that was dependent on acylation of the protein. When expressed as a recombinant protein in rapid-growing ***mycobacteria***, the 19-kDa Ag was able to deliver peptides for recognition by MHC class I-restricted T cells by a TAP-independent mechanism. Entry into the class I pathway was rapid, dependent on acylation, and could be blocked by killing the ***mycobacteria*** by heating before infection. Although the pattern

of

19-kDa trafficking was similar with different ***mycobacterial*** species, preliminary expts. suggest that class I presentation is more efficient during infection with rapid-growing ***mycobacteria*** than with the slow-growing bacillus Calmette-Guerin vaccine strain.

RE.CNT 64 THERE ARE 64 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 29 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

AN 2001:374392 BIOSIS

DN PREV200100374392
 TI Novel strategies for intervention targeted at ***mycobacterial*** persistence.
 AU ***Young, Douglas B. (1)***
 CS (1) Imperial College School of Medicine, Norfolk Place, London, W2 1PG UK
 SO Scandinavian Journal of Infectious Diseases, (2001) Vol. 33, No. 6, pp. 403-404. print.
 ISSN: 0036-5548.
 DT Article
 LA English
 SL English
 AB Effective global control of tuberculosis is likely to require intervention at multiple points in the course of infection. In addition to existing approaches based on treatment of active disease and preventive vaccination of unexposed individuals, current research on the biology of ***mycobacterial*** persistence suggests the potential for the development of novel disease-control strategies targeted at infected asymptomatic populations.

L6 ANSWER 30 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 AN 2001:336926 BIOSIS
 DN PREV200100336926
 TI Genomics: Leprosy: A degenerative disease of the genome.
 AU ***Young, Douglas (1)*** ; Robertson, Brian (1)
 CS (1) Centre for Molecular Microbiology and Infection, Imperial College, Flowers Building, London, SW7 2AZ: d.young@ic.ac.uk UK
 SO Current Biology, (15 May, 2001) Vol. 11, No. 10, pp. R381-R383. print.
 ISSN: 0960-9822.
 DT Article
 LA English
 SL English
 AB Analysis of the genome of the leprosy bacillus uncovers evidence of extensive deletion and inactivation of genes. Secluded in a specialised niche, it has discarded much of its genetic heritage, though retaining just enough to be a major human pathogen.

L6 ANSWER 31 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 2002:76794 CAPLUS
 DN 137:149571
 TI Letting the genome out of the bottle: Prospects for new drug development
 AU ***Young, Douglas***
 CS Department of Medical Microbiology, Imperial College, London, SW7 2AZ, UK
 SO Annals of the New York Academy of Sciences (2001), 953(New Vistas in Therapeutics [and] Drug-Resistant Tuberculosis), 146-150
 CODEN: ANYAA9; ISSN: 0077-8923
 PB New York Academy of Sciences
 DT Journal; General Review
 LA English
 AB A review with refs. Use of the information gained from sequencing the ***Mycobacterium*** tuberculosis genome will enable scientists to accelerate the development of reagents for improved tuberculosis control. Cloning and expressing genes encoding the enzymes involved in cell-wall biosynthesis will provide the tools for screening millions of novel compds. Cell wall inhibitors will be mainly useful in treating resistant disease, but cost factors are likely to limit the application of novel compds. in the design of new treatment regimens. More effective might be an approach to target metabolic processes that are essential even in

nondividing bacteria. A third target for drug action is elimination of latent disease through a drug that acts in synergy with the immune response.

RE.CNT 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 32 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 19
AN 2001:397712 BIOSIS
DN PREV200100397712
TI Leprosy lipid provides the key to Schwann cell entry.
AU ***Young, Douglas B. (1)***
CS (1) Dept of Infectious Diseases and Microbiology, Imperial College School
of Medicine, Norfolk Place, London, W2 1PG: d.young@ic.ac.uk UK
SO Trends in Microbiology, (February, 2001) Vol. 9, No. 2, pp. 52-54. print.
ISSN: 0966-842X.
DT Article
LA English
SL English
AB A recent study has demonstrated that the species-specific phenolic
glycolipid of ***Mycobacterium*** leprae triggers uptake into Schwann
cells by interaction with laminin-2 and the alpha-dystroglycan receptor.
This finding emphasizes the importance of lipids in the biology of
mycobacterial infection and suggests possible strategies to
combat
nerve damage in leprosy.

L6 ANSWER 33 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2003:208445 BIOSIS
DN PREV200300208445
TI Novel strategies for intervention targeted at ***mycobacterial***
persistence.
AU ***Young, Douglas B. (1)***
CS (1) Imperial College School of Medicine, Norfolk Place, London, W2 1PG, UK
UK
SO Scandinavian Journal of Infectious Diseases, (2001) No. Special Issue, pp.
41-42. print.
ISSN: 0036-5548.
DT Article
LA English
AB Effective global control of tuberculosis is likely to require intervention
at multiple points in the course of infection. In addition to existing
approaches based on treatment of active disease and preventive vaccination
of unexposed individuals, current research on the biology of
mycobacterial persistence suggests the potential for the
development of novel disease-control strategies targeted at infected
asymptomatic populations.

L6 ANSWER 34 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 20
AN 2001:227358 BIOSIS
DN PREV200100227358
TI Rapid detection of antibiotic resistance in ***mycobacterium***
tuberculosis.
AU Heym, Beate (1); Cole, Stewart; ***Young, Douglas*** ; Zhang, Ying;
Honore, Nadine; Telenti, Amalio; Bodmer, Thomas
CS (1) Ville d'Avray France

ASSIGNEE: Institut Pasteur, Paris, France; Medical Research Council, London, UK; Assistance Publique, Paris, France; Universite Pierre et Marie Curie (Paris VI), Paris, France; Universite de Berne, Berne, Switzerland

PI US 6124098 September 26, 2000

SO Official Gazette of the United States Patent and Trademark Office Patents, (Sep. 26, 2000) Vol. 1238, No. 4, pp. No Pagination. e-file.
ISSN: 0098-1133.

DT Patent

LA English

AB A nucleotide sequence encoding a katG/lacZ fusion protein is useful for assaying the enzymatic activity of the katG gene product. A process of selecting a compound that is toxic against an isoniazid-resistant mycobacterial strain comprises incubating a catalase peroxidase enzyme with an isoniazid to produce a compound that restores isoniazid susceptibility to the isoniazid-resistant mycobacterial strain.

L6 ANSWER 35 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 21

AN 2000:469662 BIOSIS

DN PREV2000000469662

TI A postgenomic approach to identification of ***Mycobacterium***
leprae-specific peptides as T-cell reagents.

AU Dockrell, Hazel M. (1); Brahmhatt, Shweta; Robertson, Brian D.; Britton, Sven; Fruth, Uli; Gebre, Negussie; Hunegnaw, Mesfin; Hussain, Rabia; Manandhar, Rakesh; Murillo, Luis; Pessolani, Maria Cristina V.; Roche, Paul; Salgado, Jorge L.; Sampaio, Elizabeth; Shahid, Firdaus; Thole, Jelle E. R.; ***Young, Douglas B.***

CS (1) Immunology Unit, Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT UK

SO Infection and Immunity, (October, 2000) Vol. 68, No. 10, pp. 5846-5855.
print.
ISSN: 0019-9567.

DT Article

LA English

SL English

AB To identify ***Mycobacterium*** leprae-specific human T-cell epitopes, which could be used to distinguish exposure to M. leprae from exposure to ***Mycobacterium*** tuberculosis or to environmental ***mycobacteria*** or from immune responses following ***Mycobacterium*** bovis BCG vaccination, 15-mer synthetic peptides were synthesized based on data from the M. leprae genome, each peptide containing three or more predicted HLA-DR binding motifs. Eighty-one peptides from 33 genes were tested for their ability to induce T-cell responses, using peripheral blood mononuclear cells (PBMC) from tuberculoid leprosy patients (n = 59) and healthy leprosy contacts (n = 53) from Brazil, Ethiopia, Nepal, and Pakistan and 20 United Kingdom blood bank donors. Gamma interferon (IFN-gamma) secretion proved more sensitive for detection of PBMC responses to peptides than did lymphocyte proliferation. Many of the peptides giving the strongest responses in leprosy donors compared to subjects from the United Kingdom, where leprosy is not endemic, have identical, or almost identical, sequences in M. leprae and M. tuberculosis and would not be suitable as diagnostic tools. Most of the peptides recognized by United Kingdom donors showed promiscuous recognition by subjects expressing differing HLA-DR types. The majority of the novel T-cell epitopes identified came from proteins not previously recognized as immune targets, many of which are cytosolic

enzymes. Fifteen of the tested peptides had gtoreq5 of 15 amino acid mismatches between the equivalent M. leprae and M. tuberculosis sequences; of these, eight gave specificities of gtoreq90% (percentage of United Kingdom donors who were nonresponders for IFN-gamma secretion), with sensitivities (percentage of responders) ranging from 19 to 47% for tuberculoid leprosy patients and 21 to 64% for healthy leprosy contacts. A pool of such peptides, formulated as a skin test reagent, could be used to monitor exposure to leprosy or as an aid to early diagnosis.

L6 ANSWER 36 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 AN 2001:116521 BIOSIS
 DN PREV200100116521
 TI A mediaeval case of lepromatous leprosy from 13-14th century Orkney, Scotland.
 AU Taylor, G. Michael (1); Widdison, Stephanie; Brown, Ivor N.; ***Young,***
 *** Douglas*** ; Molleson, Theya
 CS (1) Department of Infectious Diseases and Microbiology, Imperial College School of Medicine, Norfolk Place, St Mary's Campus, London, W2 1PG: gm.taylor@ic.ac.uk UK
 SO Journal of Archaeological Science, (December, 2000) Vol. 27, No. 12, pp. 1133-1138. print.
 ISSN: 0305-4403.
 DT Article
 LA English
 SL English
 AB Erosion in the 1960s resulted in exposure of human skeletal remains from a Norse Christian cemetery at Newark Bay, Orkney, Scotland. One set of remains showed osteological evidence of advanced lepromatous leprosy, but the absence of bones from the lower limbs precluded definitive diagnosis. The aim of the present study was to determine whether
 Mycobacterium leprae could be detected in bone extracts, as a means of confirming the diagnosis of leprosy. Bone samples were examined from the suspected leprosy case and from a second contemporary burial thought to be free of disease. DNA was amplified by polymerase chain reaction (PCR) using primers specific for a repetitive element (RLEP) characteristic of M. leprae. Additional PCR tests specific for
 Mycobacterium tuberculosis and for amelogenin (a human gene suitable for sex determination) were also applied to the samples. M. leprae DNA was detected only in the skull sample from the suspected leprosy case. The DNA sequence was identical to that found in present day isolates of M. leprae. Positive results were obtained only using a PCR reaction designed to amplify relatively short stretches of DNA (<175 bp), suggesting the microbial DNA had undergone extensive fragmentation. There was no evidence of M. tuberculosis DNA in bones from the leprosy suspect or control individual. The ability to recover ancient samples of DNA provides an opportunity to study long-term evolutionary changes that may affect the epidemiology of microbial pathogens.

L6 ANSWER 37 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 22
 AN 2000:443158 BIOSIS
 DN PREV200000443158
 TI Evaluation of human antimycobacterial immunity using recombinant reporter
 mycobacteria
 AU Kampmann, Beate (1); Gaora, Peadar O.; Snewin, Valerie A.; Gares, Marie-Pierre; ***Young, Douglas B.*** ; Levin, Michael
 CS (1) Dept. of Paediatrics, Imperial College School of Medicine, Norfolk

Place, St. Mary's Campus, London, W2 1NY UK

SO Journal of Infectious Diseases, (September, 2000) Vol. 182, No. 3, pp. 895-901. print.
ISSN: 0022-1899.

DT Article

LA English

SL English

AB A novel in vitro whole blood model was developed to study human antimycobacterial immunity. Recombinant reporter ***mycobacteria*** were used to enumerate the bacteria, and interactions between host immune cells and ***mycobacteria*** were studied using whole blood rather than cell fractions. The ability of healthy tuberculin-positive and tuberculin-negative individuals to restrict ***mycobacterial*** growth was compared. Growth of luminescent ***mycobacteria*** was significantly lower in blood samples of tuberculin-positive individuals than in blood samples of tuberculin-negative individuals (P = .005). Restricted ***mycobacterial*** growth was associated with significantly higher production of tumor necrosis factor (TNF)-alpha and interferon (IFN)-gamma (P = .01 and .004, respectively). Inhibition of the TNF-alpha and IFN-gamma response pathways by neutralizing monoclonal antibodies increased ***mycobacterial*** growth in whole blood. This model is the first functional assay in which individual variations in cell-mediated immunity are shown to correlate with differences in ability to control ***mycobacterial*** growth. It provides a new tool for studying human ***mycobactericidal*** mechanisms and, potentially, for the evaluation of improved vaccines.

L6 ANSWER 38 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 23

AN 2000:88415 BIOSIS

DN PREV200000088415

TI Identification of a ***Mycobacterium*** tuberculosis gene that enhances ***mycobacterial*** survival in macrophages.

AU Wei, Jun; Dahl, John L.; Moulder, James W.; Roberts, Esteban A.; O'Gaora, Peadar; ***Young, Douglas B.*** ; Friedman, Richard L. (1)

CS (1) Department of Microbiology and Immunology, University of Arizona College of Medicine, 1501 N. Campbell Ave., Tucson, AZ, 85724 USA

SO Journal of Bacteriology, (Jan., 2000) Vol. 182, No. 2, pp. 377-384.
ISSN: 0021-9193.

DT Article

LA English

SL English

AB Intracellular survival plays a central role in the pathogenesis of ***Mycobacterium*** tuberculosis. To identify M. tuberculosis genes required for intracellular survival within macrophages, an M. tuberculosis H37Rv plasmid library was constructed by using the shuttle vector pOLYG. This plasmid library was electroporated into ***Mycobacterium*** smegmatis 1-2c, and the transformants were used to infect the human macrophage-like cell line U-937. Because M. smegmatis does not readily survive within macrophages, any increased intracellular survival is likely due to cloned M. tuberculosis H37Rv DNA. After six sequential passages of M. smegmatis transformants through U-937 cells, one clone (p69) was enriched more than 70% as determined by both restriction enzyme and PCR analyses. p69 demonstrated significantly enhanced survival compared to that of the vector control, ranging from 2.4- to 5.3-fold at both 24 and 48 h after infection. DNA sequence analysis revealed three open reading frames (ORFs) in the insert of p69. ORF2 (1.2 kb) was the only one which

contained a putative promoter region and a ribosome-binding site. Deletion analysis of the p69 insert DNA showed that disruption of ORF2 resulted in complete loss of the enhanced intracellular survival phenotype. This gene was named the enhanced intracellular survival (eis) gene. By using an internal region of eis as a probe for Southern analysis, eis was found in the genomic DNA of various *M. tuberculosis* strains and of

****Mycobacterium**** *bovis* BCG but not in that of *M. smegmatis* or 10 other nonpathogenic ***mycobacterial*** species. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis showed that all *M. smegmatis* eis-containing constructs expressed a unique protein of 42 kDa, the predicted size of Eis. The expression of this 42-kDa protein directly correlated to the enhanced survival of *M. smegmatis* p69 in U-937 cells. These results suggest a possible role for eis and its protein product in the intracellular survival of *M. tuberculosis*.

L6 ANSWER 39 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:304819 BIOSIS
DN PREV200000304819

TI Analysis of post-translational modification of ***mycobacterial*** proteins using a cassette expression system.

AU Herrmann, Jean Louis (1); Delahay, Robin; Gallagher, Alex; Robertson, Brian; ***Young, Douglas***

CS (1) Laboratoire de Microbiologie, Centre Hospitalier, Universitaire Saint-Louis, 1 Avenue Claude-Vellefaux, 75475, Paris Cedex, 10 France

SO FEBS Letters, (May 19, 2000) Vol. 473, No. 3, pp. 358-362. print.
ISSN: 0014-5793.

DT Article

LA English

SL English

AB A recombinant expression system was developed to analyse sequence determinants involved in O-glycosylation of proteins in ***mycobacteria***. By expressing peptide sequences corresponding to known glycosylation sites within a chimeric lipoprotein construct, amino acids flanking modified threonine residues were found to have an important influence on glycosylation. The expression system was used to screen

mycobacterial sequences selected using a neural network (NetOglyc)

trained on eukaryotic O-glycoproteins. Evidence of glycosylation was obtained for eight of 11 proteins tested. The results suggest that sites involved in O-glycosylation of ***mycobacterial*** and eukaryotic proteins share similar structural features.

L6 ANSWER 40 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 24

AN 2001:71284 BIOSIS

DN PREV200100071284

TI Comparison of ****Mycobacterium**** tuberculosis genomes reveals frequent deletions in a 20 kb variable region in clinical isolates.

AU Ho, Timothy B. L. (1); Robertson, Brian D.; Taylor, G. Michael; Shaw, Rory J.; ***Young, Douglas B.***

CS (1) Department of Infectious Diseases and Microbiology, Imperial College School of Medicine, Norfolk Place, London, W2 1PG: tho@ic.ac.uk UK

SO Yeast, (December, 2000) Vol. 17, No. 4, pp. 272-282. print.
ISSN: 0749-503X.

DT Article

LA English

SL English

AB The ***Mycobacterium*** tuberculosis complex is associated with a remarkably low level of structural gene polymorphism. As part of a search for alternative forms of genetic variation that may act as a source of biological diversity in M. tuberculosis, we have identified a region of the genome that is highly variable amongst a panel of unrelated clinical isolates. Fifteen of 24 isolates examined contained one or more copies of the M. tuberculosis-specific IS6110 insertion element within this 20 kb variable region. In nine of the isolates, including the laboratory-passaged strain H37Rv, genomic deletions were identified, resulting in loss of between two and 13 genes. In each case, deletions were associated with the presence of a copy of the IS6110 element. Absence of flanking tri- or tetra-nucleotide repeats identified homologous recombination between adjacent IS6110 elements as the most likely mechanism of the deletion events. IS6110 insertion into hot-spots within the genome of M. tuberculosis provides a mechanism for generation of genetic diversity involving a high frequency of insertions and deletions.

L6 ANSWER 41 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:411011 BIOSIS
DN PREV200000411011
TI Current tuberculosis vaccine development.
AU ***Young, Douglas B. (1)***
CS (1) Department of Infectious Diseases and Microbiology, Imperial College School of Medicine, Norfolk Place, London, W2 1PG UK
SO Clinical Infectious Diseases, (June, 2000) Vol. 30, No. Supplement 3, pp. S254-S256. print.
ISSN: 1058-4838.

DT Article
LA English
SL English

AB Information derived from the complete genome sequence of
of ***Mycobacterium*** tuberculosis makes it possible to develop a range
new vaccine candidates. Strategies currently under investigation include construction of whole cell live attenuated ***mycobacterial*** vaccines, as well as the use of individual antigens delivered by a variety of subunit vaccination procedures. Fundamental questions associated with the rational design, preclinical testing, and future application of new tuberculosis vaccines are reviewed.

L6 ANSWER 42 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 25
AN 2000:99110 BIOSIS
DN PREV200000099110
TI Three pathways for trehalose biosynthesis in ***mycobacteria***
AU De Smet, Koen A. L.; Weston, Anthony; Brown, Ivor N.; ***Young, Douglas***
*** B.*** ; Robertson, Brian D. (1)

CS (1) Department of Infectious Diseases and Microbiology, Imperial College School of Medicine, Norfolk Place, St Mary's Campus, London, W2 1PG UK
SO Microbiology (Reading), (Jan., 2000) Vol. 146, No. 1, pp. 199-208.
ISSN: 1350-0872.

DT Article
LA English
SL English

AB Trehalose is present as a free disaccharide in the cytoplasm of
mycobacteria and as a component of cell-wall glycolipids

implicated in tissue damage associated with ***mycobacterial*** infection. To obtain an overview of trehalose metabolism, we analysed data from the ***Mycobacterium*** tuberculosis genome project and identified ORFs with homology to genes encoding enzymes from three trehalose biosynthesis pathways previously characterized in other bacteria. Functional assays using ***mycobacterial*** extracts and recombinant enzymes derived from these ORFs demonstrated that ***mycobacteria*** can produce trehalose from glucose 6-phosphate and UDP-glucose (the OtsA-OtsB pathway) from glycogen-like alpha(1 fvdarw 4)-linked glucose polymers (the TreY-TreZ pathway) and from maltose (the TreS pathway). Each of the pathways was found to be active in both rapid-growing ***Mycobacterium*** smegmatis and slow-growing ***Mycobacterium*** bovis BCG. The presence of a disrupted treZ gene in ***Mycobacterium*** leprae suggests that this pathway is not functional in this organism. The presence of multiple biosynthetic pathways indicates that trehalose plays an important role in ***mycobacterial*** physiology.

L6 ANSWER 43 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 AN 2001:84297 BIOSIS
 DN PREV200100084297
 TI The role of TGFbeta1 in the pathology of ***mycobacterial*** infection.
 AU Roe, Thomas (1); Lukey, Pauline (1); Muller, Ingrid (1); ***Young,***
 *** Douglas (1)***
 CS (1) Department of Infectious Diseases and Microbiology, Division of Investigative Science, Imperial College School of Medicine, Norfolk Place, London, W2 1PG UK
 SO Immunology, (December, 2000) Vol. 101, No. Supplement 1, pp. 113. print. Meeting Info.: Annual Congress of the British Society for Immunology Harrogate, UK December 05-08, 2000 British Society for Immunology . ISSN: 0019-2805.
 DT Conference
 LA English
 SL English

L6 ANSWER 44 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 26
 AN 1999:808585 CAPLUS
 DN 132:44952
 TI Method of identifying compounds that regulate the binding of ***Mycobacterium*** tuberculosis sigF to M. tuberculosis orfX
 IN Bishai, William R.; ***Young, Douglas B.*** ; Zhang, Ying; Demaio, James
 PA Johns Hopkins University, USA
 SO U.S., 27 pp., Cont.-in-part of U.S. 5,824,546.
 CODEN: USXXAM
 DT Patent
 LA English
 FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6004764	A	19991221	US 1997-826390	19970409
	US 5700925	A	19971223	US 1996-622353	19960327
	US 5824546	A	19981020	US 1996-622352	19960327
	CA 2249208	AA	19971002	CA 1997-2249208	19970327
PRAI	US 1996-622352	A2	19960327		
	US 1996-622353	A2	19960327		

AB SigF is a gene that controls M. tuberculosis latency. A diagnostic test for latent tuberculosis involves detecting M. tuberculosis sigF in clin. specimens. Two genes orfX and orfY regulate sigF expression and sigF activity. M. tuberculosis sigF, orfX, and orfY are used in screening methods for potential therapeutic agents which regulate the growth of M. tuberculosis.

RE.CNT 56 THERE ARE 56 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 45 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 27

AN 1999:125729 CAPLUS

DN 130:178321

TI Nucleic acid probes, sequences and methods for detecting
Mycobacterium tuberculosis resistant to isoniazid

IN Heym, Beate; Cole, Stewart T.; ***Young, Douglas B.*** ; Zhang, Ying
PA Institut Pasteur, Fr.

SO U.S., 46 pp., Cont.-in-part of U.S. Ser. No. 29,655, abandoned.
CODEN: USXXAM

DT Patent

LA English

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5871912	A	19990216	US 1995-459499	19950602
	JP 07059595	A2	19950307	JP 1992-312596	19921009
	JP 3408564	B2	20030519		
	JP 2003225097	A2	20030812	JP 2002-374077	19921009
PRAI	US 1992-875940	B2	19920430		
	US 1992-929206	A2	19920814		
	US 1993-29655	B2	19930311		
	JP 1992-312596	A3	19921009		

AB Multi-drug resistant strains of ***Mycobacterium*** tuberculosis represent a considerable threat to public health worldwide. Resistance to isoniazid (INH), a key component of anti-tuberculosis regimens, is often assocd. with loss of catalase activity and virulence. The katG gene, encoding HPI catalase-peroxidase, mediates INH-sensitivity and the high level resistance encountered clin. may be due to deletions, insertions or point mutations which reduce or eliminate the expression of the catalase gene in the chromosomal region encompassing katG. INH-resistant strains of ***Mycobacterium*** tuberculosis are detected by nucleic acid hybridization with a unique nucleic acid sequence or by amplification techniques.

L6 ANSWER 46 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 28

AN 1999:446719 BIOSIS

DN PREV199900446719

TI Assessment of immunity to ***mycobacterial*** infection with luciferase reporter constructs.

AU Snewin, Valerie A. (1); Gares, Marie-Pierre; Gaora, Peadar O.; Hasan, Zahra; Brown, Ivor N.; ***Young, Douglas B.***

CS (1) Department of Infectious Diseases and Microbiology, Imperial College School of Medicine, Norfolk Place, St. Mary's Campus, London, W2 1PG UK

SO Infection and Immunity, (Sept., 1999) Vol. 67, No. 9, pp. 4586-4593.
ISSN: 0019-9567.

DT Article

LA English

SL English

AB Protective immunity to ***mycobacterial*** infection is incompletely understood but probably involves the coordinated interaction of multiple cell types and cytokines. With the aim of developing assays that might provide a surrogate measure of protective immunity, we have investigated the use of recombinant ***mycobacteria*** carrying luciferase reporter enzymes to assess the effectiveness of antimycobacterial immunity in model systems. Measurement of luminescence was shown to provide a rapid and simple alternative to the counting of CFU as a means of monitoring ***mycobacterial*** viability. We describe optimization of a luciferase reporter strain of ***Mycobacterium*** tuberculosis and demonstrate its application for the study of ***mycobacterial*** interactions with host cells in tissue culture and the rapid assessment of vaccine efficacy in a murine model.

L6 ANSWER 47 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 29

AN 2000:27384 BIOSIS

DN PREV200000027384

TI Alteration of a single amino acid residue reverses fosfomycin resistance of recombinant MurA from ***Mycobacterium*** tuberculosis.

AU De Smet, Koen A.L.; Kempell, Karen E.; Gallagher, Alex; Duncan, Ken; ***Young, Douglas B. (1)***

CS (1) Department of Infectious Diseases and Microbiology, Imperial College School of Medicine, Norfolk Place, St Mary's Campus, London, W2 1PG UK

SO Microbiology (Reading), (Nov., 1999) Vol. 145, No. 11, pp. 3177-3184. ISSN: 1350-0872.

DT Article

LA English

SL English

AB ***Mycobacterium*** tuberculosis has innate resistance to a range of broadspectrum antimicrobial agents. This may in part reflect the relative impermeability of the ***mycobacterial*** cell wall, but additional specific mechanisms may also be important. In the case of fosfomycin, it has been suggested that a key difference in the active site of the M. tuberculosis MurA enzyme might confer resistance. In Escherichia coli, fosfomycin covalently binds to a cysteine normally involved in the enzymic activity, while protein alignments predict an aspartate of this position in the M. tuberculosis MurA. In the present study, it is demonstrated that the wild-type M. tuberculosis MurA is indeed resistant to fosfomycin, and that it becomes sensitive following replacement of the aspartate residue in position 117 by a cysteine. In addition, the study illustrates the use of an inducible expression system in ***mycobacteria*** to allow functional characterization of an M. tuberculosis enzyme that is unstable during constitutive expression.

L6 ANSWER 48 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 30

AN 1999:237620 BIOSIS

DN PREV199900237620

TI Construction and murine immunogenicity of recombinant Bacille Calmette Guerin vaccines expressing the B subunit of Escherichia coli heat labile enterotoxin.

AU Hayward, Christopher M. M.; O'Gaora, Peadar; ***Young, Douglas B.*** ; Griffin, George E.; Thole, Jelle; Hirst, Timothy R.; Castello-Branco, Luiz R. R.; Lewis, David J. M. (1)

CS (1) Division of Infectious Diseases, Saint George's Hospital Medical

School, London, SW17 0RE UK

SO Vaccine, (March, 1999) Vol. 17, No. 9-10, pp. 1272-1281.
ISSN: 0264-410X.

DT Article

LA English

SL English

AB Three recombinant strains of ***Mycobacterium*** bovis Bacille Calmette Guerin (rBCG) were prepared in which the immunogenic B subunit of human Escherichia coli heat labile enterotoxin (LT-Bh) was expressed either as a cytoplasm protein, a cell wall associated lipoprotein or a secreted protein. Intraperitoneal immunisation of mice with these rBCG induced IgG and IgA antibodies to LT-Bh and shifted the serum IgG subclass response to subsequent challenge with purified LT-Bh from IgG1 to an IgG2a. Oral administration of recombinant BCG induced mucosal and serum IgA antibodies to LT-Bh which peaked four months after immunisation. Antibody responses were greater when LT-Bh was expressed as a secreted protein or lipoprotein rather than in the cytoplasm. Oral vaccination with recombinant BCG may be an effective approach, particularly to induce mucosal IgA and prime for a serum TH1 recall response.

L6 ANSWER 49 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2000:903472 CAPLUS

DN 135:59771

TI ***Mycobacterial*** antigens

AU Thole, Jelle; Janssen, Riny; ***Young, Douglas***

CS Department of Infectious Diseases & Microbiology, Imperial College School of Medicine, London, UK

SO Mycobacteria (1999), 356-370. Editor(s): Ratledge, Colin; Dale, Jeremy. Publisher: Blackwell Science Ltd., Oxford, UK.
CODEN: 69ATXF

DT Conference; General Review

LA English

AB A review with 102 refs. Topics discussed include the identification and characterization of the antigens; the structure and function of cytoplasmic antigens, cell-wall antigens, and secreted antigens; and future advances.

RE.CNT 102 THERE ARE 102 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 50 OF 121 USPATFULL on STN

AN 1998:159697 USPATFULL

TI Rapid detection of antibiotic resistance in ***mycobacterium*** tuberculosis

IN Heym, Beate, Ville d'Avray, France
Cole, Stewart, Clamart, France
Young, Douglas, Ruislip, United Kingdom
Zhang, Ying, London, United Kingdom
Honore, Nadine, Colombes, France
Telenti, Amalio, Gerzensee, Switzerland
Bodmer, Thomas, Ersigen, Switzerland

PA Institut Pasteur, Paris, France (non-U.S. corporation)

PI US 5851763 19981222
WO 9322454 19931111

AI US 1994-313185 19941012 (8)
WO 1993-EP1063 19930430
19950509 PCT 371 date
19950509 PCT 102(e) date

PRAI FR 1992-11098 19920917
FR 1993-4545 19930416
DT Utility
FS Granted
EXNAM Primary Examiner: Horlick, Kenneth R.; Assistant Examiner: Tung, Joyce
LREP Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.
CLMN Number of Claims: 21
ECL Exemplary Claim: 1
DRWN 30 Drawing Figure(s); 26 Drawing Page(s)
LN.CNT 2597

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A process for the detection of resistance to an antibiotic in a
mycobacterium comprises detecting a mutation in a gene selected
from the group consisting of the katG gene or fragment thereof, the rpoB
gene or fragment thereof, and the rpsI gene or fragment thereof. The
process is useful for detecting in vitro the presence of nucleic acids
of a ***Mycobacterium*** tuberculosis resistant to isoniazid.

L6 ANSWER 51 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1998:328383 BIOSIS
DN PREV199800328383
TI Molecular approaches in ***Mycobacterium*** tuberculosis and other
infections caused by ***Mycobacterium*** species.
AU Goyal, Madhu (1); ***Young, Douglas***
CS (1) Imperial Coll. Sch. Med., St. Mary's Campus, London UK
SO Woodford, N. [Editor]; Johnson, A. P. [Editor]. Methods in Molecular
Medicine, (1998) Vol. 15, pp. 157-190. Methods in Molecular Medicine;
Molecular bacteriology: Protocols and clinical applications.
Publisher: Humana Press Inc. Suite 808, 999 Riverview Drive, Totowa, New
Jersey 07512, USA.
ISBN: 0-89603-498-4.
DT Book
LA English

L6 ANSWER 52 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 31
AN 1999:8459 BIOSIS
DN PREV199900008459
TI Molecular cloning and characterization of Tap, a putative multidrug efflux
pump present in ***Mycobacterium*** fortuitum and
Mycobacterium tuberculosis.
AU Ainsa, Jose A.; Blokpoel, Marian C. J.; Otal, Isabel; ***Young,
Douglas***
*** B.*** ; De Smet, Koen A. L.; Martin, Carlos (1)
CS (1) Dep. Microbiol. Med. Preventiva Salud Publ., Univ. Zaragoza, C/Domingo
Miral s/n, 50009 Zaragoza Spain
SO Journal of Bacteriology, (Nov., 1998) Vol. 180, No. 22, pp. 5836-5843.
ISSN: 0021-9193.
DT Article
LA English
AB A recombinant plasmid isolated from a ***Mycobacterium*** fortuitum
genomic library by selection for gentamicin and 2-N'-ethylnetilmicin
resistance conferred low-level aminoglycoside and tetracycline resistance
when introduced into M. smegmatis. Further characterization of this
plasmid allowed the identification of the M. fortuitum tap gene. A
homologous gene in the M. tuberculosis H37Rv genome has been identified.
The M. tuberculosis tap gene (Rv1258 in the annotated sequence of the M.

tuberculosis genome) was cloned and conferred low-level resistance to tetracycline when introduced into *M. smegmatis*. The sequences of the putative Tap proteins showed 20 to 30% amino acid identity to, membrane efflux pumps of the major facilitator superfamily (MFS), mainly tetracycline and macrolide efflux pumps, and to other proteins of unknown function but with similar antibiotic resistance patterns. Approximately 12 transmembrane regions and different sequence motifs characteristic of the MFS proteins also were detected. In the presence of the protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), the levels of resistance to antibiotics conferred by plasmids containing the tap genes were decreased. When tetracycline accumulation experiments were carried out with the *M. fortuitum* tap gene, the level of tetracycline accumulation was lower than that in control cells but was independent of the presence of CCCP. We conclude that the Tap proteins of the opportunistic organism *M. fortuitum* and the important pathogen *M. tuberculosis* are probably proton-dependent efflux pumps, although we cannot exclude the possibility that they act as regulatory proteins.

- L6 ANSWER 53 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 32
AN 1998:393348 BIOSIS
DN PREV199800393348
TI An epitope delivery system for use with recombinant ***mycobacteria***
- AU Hetzel, Charlotte; Janssen, Riny; Ely, Sarah J.; Kristensen, Nanna M.;
Bunting, Karen; Cooper, Jonathan B.; Lamb, Jonathan R.; ***Young,***
*** Douglas B.*** ; Thole, Jelle E. R. (1)
CS (1) TNO Prevention Health, Zernikedreef 9, P.O. Box 2215, 2301 CE Leiden
Netherlands
SO Infection and Immunity, (Aug., 1998) Vol. 66, No. 8, pp. 3643-3648.
ISSN: 0019-9567.
DT Article
LA English
AB We have developed a novel epitope delivery system based on the insertion
of peptides within a permissive loop of a bacterial superoxide dismutase
molecule. This system allowed high-level expression of heterologous
peptides in two ***mycobacterial*** vaccine strains,
Mycobacterium bovis bacille Calmette-Guerin (BCG) and
Mycobacterium vaccae. The broader application of the system was
analyzed by preparation of constructs containing peptide epitopes from a
range of infectious agents and allergens. We report detailed
characterization of the immunogenicity of one such construct, in which an
epitope from the Der p1 house dust mite allergen was expressed in *M.*
vaccae. The construct was able to stimulate T-cell hybridomas specific for
Der p1, and it induced peptide-specific gamma interferon responses when
used to immunize naive mice. This novel expression system demonstrates new
possibilities for the use of ***mycobacteria*** as vaccine delivery
vehicles.
- L6 ANSWER 54 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 33
AN 1998:404277 BIOSIS
DN PREV199800404277
TI Molecular cloning and functional analysis of a novel tetracycline
resistance determinant, tet(V), from ***Mycobacterium*** smegmatis.
AU De Rossi, Edda; Blokpoel, Marian C. J.; Cantoni, Rita; Branzoni, Manuela;
Riccardi, Giovanna; ***Young, Douglas B.*** ; De Smet, Koen A. L.;

Ciferri, Orio (1)
 CS (1) Dep. Genet. Microbiol., via Abbiategrasso 207, 27100 Pavia Italy
 SO Antimicrobial Agents and Chemotherapy, (Aug., 1998) Vol. 42, No. 8, pp. 1931-1937.
 ISSN: 0066-4804.
 DT Article
 LA English
 AB The nucleotide sequence and mechanism of action of a tetracycline resistance gene from ***Mycobacterium*** smegmatis were determined. Analysis of a 2.2-kb sequence fragment showed the presence of one open reading frame, designated tet(V), encoding a 419-amino-acid protein (molecular weight, 44,610) with at least 10 transmembrane domains. A database search showed that the gene is homologous to membrane-associated antibiotic efflux pump proteins but not to any known tetracycline efflux pumps. The steady-state accumulation level of tetracycline by *M. smegmatis* harboring a plasmid carrying the tet(V) gene was about fourfold lower than that of the parental strain. Furthermore, the energy uncoupler carbonyl cyanide m-chlorophenylhydrazone blocked tetracycline efflux in deenergized cells. These results suggest that the tet(V) gene codes for a drug antiporter which uses the proton motive force for the active efflux of tetracycline. By primer-specific amplification the gene appears to be restricted to *M. smegmatis* and *M. fortuitum*.

L6 ANSWER 55 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 34
 AN 1998:134139 BIOSIS
 DN PREV199800134139
 TI Engineering a change in metal-ion specificity of the iron-dependent superoxide dismutase from ***Mycobacterium*** tuberculosis X-ray structure analysis of site-directed mutants.
 AU Bunting, Karen; Cooper, Jon B. (1); Badasso, Mohammed O.; Tickle, Ian J.; Newton, Melanie; Wood, Steve P.; Zhang, Ying; ***Young, Douglas***
 CS (1) Div. Biochem. and Molecular Biol., Sch. Biol. Sci., Univ. Southampton, Bassett Crescent East, Southampton, SO16 7PX UK
 SO European Journal of Biochemistry, (Feb., 1998) Vol. 251, No. 3, pp. 795-803.
 ISSN: 0014-2956.
 DT Article
 LA English
 AB We have refined the X-ray structures of two site-directed mutants of the iron-dependent superoxide dismutase (SOD) from ***Mycobacterium*** tuberculosis. These mutations which affect residue 145 in the enzyme (H145Q and H145E) were designed to alter its metal-ion specificity. This residue is either Gln or His in homologous SOD enzymes and has previously been shown to play a role in active-site interactions since its side-chain helps to coordinate the metal ion via a solvent molecule which is thought to be a hydroxide ion. The mutations were based on the observation that in the closely homologous manganese dependent SOD from ***Mycobacterium*** leprae, the only significant difference from the *M. tuberculosis* SOD within 10 Å of the metal-binding site is the substitution of Gln for His at position 145. Hence an H145Q mutant of the *M. tuberculosis* (TB) SOD was engineered to investigate this residue's role in metal ion dependence and an isosteric H145E mutant was also expressed. The X-ray structures of the H145Q and H145E mutants have been solved at resolutions of 4.0 Å and 2.5 Å respectively, confirming that neither mutation has any gross effects on the conformation of the enzyme or the structure of the active site. The residue substitutions are accommodated in the enzyme's three-dimensional

structure by small local conformational changes. Peroxide inhibition experiments and atomic absorption spectroscopy establish surprisingly the H145E mutant SOD has manganese bound to it whereas the H145Q mutant SOD retains iron as the active-site metal. This alteration in metal specificity may reflect on the preference of manganese ions for anionic ligands.

- L6 ANSWER 56 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 35
AN 1999:87674 BIOSIS
DN PREV199900087674
TI Approaches to combat tuberculosis.
AU ***Young, Douglas B. (1)*** ; Robertson, Brian D.
CS (1) Dep. Infect. Dis. Microbiol., Imperial Coll. Sch. Med., St Mary's
Campus, Norfolk Place, London W2 1PG UK
SO Current Opinion in Biotechnology, (Dec., 1998) Vol. 9, No. 6, pp. 650-652.
ISSN: 0958-1669.
DT Article
LA English
- L6 ANSWER 57 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1998:372863 BIOSIS
DN PREV199800372863
TI Childhood tuberculosis: Advances in immunopathogenesis, treatment and
prevention.
AU Kampman, Beate (1); ***Young, Douglas***
CS (1) Dep. Pediatr., Imperial Coll. Sch. Med. St. Mary's, 7th Floor, QEQM
Build., St. Mary's Hosp., South Wharf Road, London W2 1NY UK
SO Current Opinion in Infectious Diseases, (June, 1998) Vol. 11, No. 3, pp.
331-335.
ISSN: 0951-7375.
DT General Review
LA English
- L6 ANSWER 58 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1998:348426 BIOSIS
DN PREV199800348426
TI Leprosy: A post-elimination research agenda (The future role of biomedical
research in leprosy) (Addis Ababa, Ethiopia; February 27-28, 1998).
AU ***Young, Douglas (1)***
CS (1) Imperial Coll. Sch. Med., Norfolk Place, London W2 1PG UK
SO Trends in Microbiology, (June, 1998) Vol. 6, No. 6, pp. 217-218.
ISSN: 0966-842X.
DT Article
LA English
- L6 ANSWER 59 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1999:208278 CAPLUS
DN 131:40283
TI Molecular approaches in ***Mycobacterium*** tuberculosis and other
infections caused by ***Mycobacterium*** species
AU Goyal, Madhu; ***Young, Douglas***
CS Imperial College School of Medicine, London, UK
SO Methods in Molecular Medicine (1998), 15 (Molecular Bacteriology), 157-190
CODEN: MMMEFN
PB Humana Press Inc.
DT Journal

LA English
AB This article describes the progress in the development of different methods, including mol. techniques, used for the diagnosis and epidemiol. of tuberculosis.

RE.CNT 137 THERE ARE 137 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 60 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1997:650293 CAPLUS

DN 127:315573

TI ***Mycobacterium*** tuberculosis gene sigF and .sigma.F factor, gene orfX and orfY sequences, and tuberculosis diagnosis and antitubercular agent screening

IN Demaio, James; ***Young, Douglas B.*** ; Bishai, William R.; Zhang, Ying

PA Johns-Hopkins University, USA

SO PCT Int. Appl., 72 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9735611	A1	19971002	WO 1997-US3457	19970327
	W: AU, CA, JP				
	RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	US 5700925	A	19971223	US 1996-622353	19960327
	US 5824546	A	19981020	US 1996-622352	19960327
	CA 2249208	AA	19971002	CA 1997-2249208	19970327
	AU 9725802	A1	19971017	AU 1997-25802	19970327
	AU 732858	B2	20010503		
	EP 910403	A1	19990428	EP 1997-917506	19970327
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 2000508525	T2	20000711	JP 1997-534404	19970327
PRAI	US 1996-622352	A	19960327		
	US 1996-622353	A	19960327		
	WO 1997-US3457	W	19970327		

AB Gene sigF .sigma. factor regulates ***Mycobacterium*** tuberculosis latency. A diagnostic test for latent tuberculosis involves detecting M. tuberculosis sigF in clin. specimens. Genes orfX and orfY regulate sigF expression and sigF activity. M. tuberculosis sigF, orfX and orfY are used in screening methods for potential therapeutic agents which regulate the growth of M. tuberculosis.

L6 ANSWER 61 OF 121 USPATFULL on STN

AN 97:120735 USPATFULL

TI DNA encoding stationary phase, stress response sigma factor from ***Mycobacterium*** tuberculosis

IN Bishai, William R., Baltimore, MD, United States

Young, Douglas B. , London, United Kingdom

Zhang, Ying, Baltimore, MD, United States

DeMaio, James, Tacoma, WA, United States

PA The Johns Hopkins University, Baltimore, MD, United States (U.S. corporation)

PI US 5700925 19971223

AI US 1996-622353 19960327 (8)

DT Utility
FS Granted
EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Swartz, Rodney P.
LREP Cushman Darby & Cushman IP Group of Pillsbury Madison & Sutro
CLMN Number of Claims: 6
ECL Exemplary Claim: 2
DRWN 6 Drawing Figure(s); 5 Drawing Page(s)
LN.CNT 858

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB SigF is a gene that controls M. tuberculosis latency. A diagnostic test for latent tuberculosis involves detecting M. tuberculosis sigF in clinical specimens. A tuberculosis vaccine includes a M. tuberculosis strain with a mutation which disrupts the reading frame of its sigF gene.

L6 ANSWER 62 OF 121 USPATFULL on STN

AN 97:44897 USPATFULL

TI Rapid detection of isoniazid resistance in ***mycobacterium*** tuberculosis probes for selecting nucleic acid encoding isoniazid resistance, and methods and kits

IN Heym, Beate, Paris, France

Cole, Stewart T., Clamart, France

Young, Douglas B., Middlesex, United Kingdom

Zhang, Ying, London, England

PA Institut Pasteur, Paris, France (non-U.S. corporation)

Medical Research Council, London, United Kingdom (non-U.S. corporation)

Assistance Publique, Paris, France (non-U.S. corporation)

Universite Paris VI, Paris, France (non-U.S. corporation)

PI US 5633131 19970527

AI US 1992-929206 19920814 (7)

RLI Continuation-in-part of Ser. No. US 1992-875940, filed on 30 Apr 1992, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Jones, W. Gary; Assistant Examiner: Marschel, Ardin H.

LREP Finnegan, Henderson, Farabow, Garrett & Dunner

CLMN Number of Claims: 22

ECL Exemplary Claim: 1

DRWN 12 Drawing Figure(s); 7 Drawing Page(s)

LN.CNT 899

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Multi-drug resistant strains of ***Mycobacterium*** tuberculosis represent a considerable threat to public health worldwide. Resistance to isoniazid (INH), a key component of anti-tuberculosis regimens, is often associated with loss of catalase activity and virulence. The katG gene, encoding HPI catalase-peroxidase, mediates INH-sensitivity and that the high level resistance encountered clinically may be due to deletions, insertions or point mutations which reduce or eliminate the expression of the catalase gene in the chromosomal region encompassing katG. INH-resistant strains of ***Mycobacterium*** tuberculosis are detected by nucleic acid hybridization with a unique nucleic acid sequence or by amplification techniques.

L6 ANSWER 63 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 36

AN 1997:251401 BIOSIS

DN PREV199799550604
 TI Production of tumor necrosis factor and nitric oxide by macrophages infected with live and dead ***mycobacteria*** and their suppression by an interleukin-10-secreting recombinant.
 AU Marshall, Ben G.; Chambers, Mark A. (1); Wangoo, Arun; Shaw, Rory J.; ***Young, Douglas B.***
 CS (1) Bacteriol. Dep., Veterinary Lab. Agency, New Haw, Addlestone, Surrey KT15 3NB UK
 SO Infection and Immunity, (1997) Vol. 65, No. 5, pp. 1931-1935. ISSN: 0019-9567.
 DT Article
 LA English
 AB We have analyzed ***mycobacterium*** -induced cytokine secretion in the J774A.1 macrophage-like cell line. Tumor necrosis factor alpha (TNF-alpha) was preferentially induced by live organisms, both slow and rapid growing. Expression of interleukin-10 by a recombinant strain of ***Mycobacterium*** smegmatis caused reduced production of TNF-alpha and nitric oxide during the early stages of infection.

L6 ANSWER 64 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 37
 AN 1997:250417 BIOSIS
 DN PREV199799549620
 TI Induction of a type 1 immune response to a recombinant antigen from ***Mycobacterium*** tuberculosis expressed in ***Mycobacterium*** vaccae.
 AU Abou-Zeid, Christiane (1); Gares, Marie-Pierre; Inwald, Jacqueline; Janssen, Riny; Zhang, Ying; ***Young, Douglas B.*** ; Hetzel, Charlotte; Lamb, Jonathan R.; Baldwin, Susan L.; Orme, Ian M.; Yermeev, Vladimir; Nikonenko, Boris V.; Apt, Alexander S.
 CS (1) Dep. Med. Microbiol., Imperial Coll. Sch. Med. St. Mary's Norfolk Place, London W2 1PG UK
 SO Infection and Immunity, (1997) Vol. 65, No. 5, pp. 1856-1862. ISSN: 0019-9567.
 DT Article
 LA English
 AB A 19-kDa lipoprotein from ***Mycobacterium*** tuberculosis was expressed as a recombinant antigen in the nonpathogenic ***mycobacterial*** host strain M. vaccae. Immunization of mice with the recombinant M. vaccae resulted in induction of a strong type 1 immune response to the 19-kDa antigen, characterized by immunoglobulin G2a (IgG2a) antibodies and gamma interferon (IFN-gamma) production by splenocytes. Immunization with the same antigen in incomplete Freund's adjuvant induced a strong IgG1 response with only low levels of IFN-gamma. Subsequent intravenous and aerosol challenges of immunized mice with virulent M. tuberculosis demonstrated no evidence of protection associated with the response to the 19-kDa antigen; in fact, the presence of the recombinant 19-kDa antigen abrogated the limited protection conferred by M. vaccae (vector control). The recombinant M. vaccae system is a convenient approach to induction of type 1 responses to M. tuberculosis antigens. However, the unexpected reduction in protective efficacy of M. vaccae expressing the 19-kDa antigen highlights the complexity of testing recombinant subunit vaccines and the need for a better understanding of the immune mechanisms required for effective vaccination against tuberculosis.

L6 ANSWER 65 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1997:119413 CAPLUS
 DN 126:210700
 TI Differential responses to challenge with live and dead
 Mycobacterium bovis Bacillus Calmette-Guerin
 AU Chambers, Mark A.; Marshall, Ben G.; Wangoo, Arun; Bune, Alison; Cook, H.
 Terry; Shaw, Rory J.; ***Young, Douglas B.***
 CS School Medicine St. Mary's, Imperial College, London, W2 1PG, UK
 SO Journal of Immunology (1997), 158(4), 1742-1748
 CODEN: JOIMA3; ISSN: 0022-1767
 PB American Association of Immunologists
 DT Journal
 LA English
 AB Bacillus Calmette-Guerin (BCG) vaccination has been shown to protect
 against challenge with virulent ***Mycobacterium*** tuberculosis in a
 range of exptl. animal models; in each case, protective efficacy requires
 vaccination with live bacteria. With the goal of moving to a new
 generation of safer, nonliving vaccines, efforts have been made to
 identify the factors that det. the efficacy of the live vaccination. The
 authors show that injection of live, but not dead, BCG induces localized
 swelling in the mouse, footpad model. Live and dead bacteria induce
 similar responses during the first week after vaccination as detd. by
 immunohistochem. anal. of the site of injection and of the draining lymph
 node. The subsequent differential response is characterized by migration
 of acid-fast bacilli to the draining lymph node in the case of the live
 vaccine. This is accompanied by an increase in mononuclear cells in the
 lymph node and by expression of inducible nitric oxide synthase both in
 the lymph node and at the site of injection. The ability of the bacteria
 to migrate to the lymph node may be an important element in the efficacy
 of live BCG vaccination.

L6 ANSWER 66 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 38
 AN 1997:320836 BIOSIS
 DN PREV199799611324
 TI Isolation and characterization of the ***mycobacterial*** phagosome:
 Segregation from the endosomal/lysosomal pathway.
 AU Hasan, Zahra; Schlax, Claudia; Lefkovits, Lotte Vv Kuhn; ***Young,***
 *** Douglas*** ; Thole, Jelle; Pieters, Jean (1)
 CS (1) Basel Inst. Immunol., Grenzacherstr. 487, CH-4005 Basel Switzerland
 SO Molecular Microbiology, (1997) Vol. 24, No. 3, pp. 545-553.
 ISSN: 0950-382X.
 DT Article
 LA English
 AB ***Mycobacteria*** have the ability to persist within host phagocytes,
 and their success as intracellular pathogens is thought to be related to
 the ability to modify their intracellular environment. After entry into
 phagocytes, ***mycobacteria*** -containing phagosomes acquire markers
 for the endosomal pathway, but do not fuse with lysosomes. The molecular
 machinery that is involved in the entry and survival of
 mycobacteria in host cells is poorly characterized. Here we
 describe the use of organelle electrophoresis to study the uptake of
 Mycobacterium bovis bacille Calmette Guerin (BCG) into murine
 macrophages. We demonstrate that live, but not dead, ***mycobacteria***
 occupy a phagosome that can be physically separated from endosomal/
 lysosomal compartments. Biochemical analysis of purified

mycobacterial phagosomes revealed the absence of endosomal/lysosomal markers LAMP-1 and beta-hexosaminidase. Combining subcellular fractionation with two-dimensional gel electrophoresis, we found that a set of host proteins was present in phagosomes that were absent from endosomal/lysosomal compartments. The residence of ***mycobacteria*** in compartments outside the endosomal/lysosomal system may explain their persistence inside host cells and their sequestration from immune recognition. Furthermore, the approach described here may contribute to an improved understanding of the molecular mechanisms that determine the intracellular fate of ***mycobacteria*** during infection.

L6 ANSWER 67 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1997:568048 CAPLUS
 DN 127:188119
 TI Isolation and characterization of the ***mycobacterial*** phagosome: segregation from the endosomal/lysosomal pathway. [Erratum to document cited in CA127:78472]
 AU Hasan, Zahra; Schlax, Claudia; Kuhn, Lotte; Lefkovits, Ivan; ***Young,***
 *** Douglas*** ; Thole, Jelle; Pieters, Jean
 CS Dep. Med. Microbiol., Imperial Coll. Sch. Med. St. Mary's, London, W2 1PG, UK
 SO Molecular Microbiology (1997), 25(2), 427
 CODEN: MOMIEE; ISSN: 0950-382X
 PB Blackwell
 DT Journal
 LA English
 AB This article was published in Mol Microbiol(1997)24(3), 545-553. Dr Jean Pieters' affiliation should have included the Netherlands Cancer Institute, where part of the work that is presented was carried out.

L6 ANSWER 68 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 AN 1997:118695 BIOSIS
 DN PREV199799425198
 TI TB research: Entering the post-genomic era.
 AU ***Young, Douglas B.***
 CS Imperial Coll. Sch. Med. St. Mary's, Norfolk Place, London W2 1PG UK
 SO Molecular Medicine Today, (1997) Vol. 3, No. 1, pp. 6-7.
 ISSN: 1357-4310.
 DT Journal; Article
 LA English

L6 ANSWER 69 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 AN 1996:333050 BIOSIS
 DN PREV199699055406
 TI Designing a vaccine for tuberculosis.
 AU Malin, Adam S. (1); ***Young, Douglas B.***
 CS (1) Dep. Clinical Sci., London Sch. Hygiene Tropical Med., London WC1E 7HT UK
 SO British Medical Journal, (1996) Vol. 312, No. 7045, pp. 1495.
 ISSN: 0959-8138.
 DT Editorial
 LA English

L6 ANSWER 70 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 39
 AN 1996:225556 BIOSIS

DN PREV199698781685
 TI A stationary-phase stress-response sigma factor from ***Mycobacterium*** tuberculosis.
 AU Demaio, James; Zhang, Ying; Ko, Chiew; ***Young, Douglas B.*** ; Bishai, William R. (1)
 CS (1) Dep. Mol. Microbiol. Immunol., Johns Hopkins Sch. Hygiene, Public Health, 615 North Wolfe St., Baltimore, MD 21205 USA
 SO Proceedings of the National Academy of Sciences of the United States of America, (1996) Vol. 93, No. 7, pp. 2790-2794. ISSN: 0027-8424.
 DT Article
 LA English
 AB Alternative RNA polymerase sigma factors are a common means of coordinating gene regulation in bacteria. Using PCR amplification with degenerate primers, we identified and cloned a sigma factor gene, sigF, from ***Mycobacterium*** tuberculosis. The deduced protein encoded by sigF shows significant similarity to SigF sporulation sigma factors from Streptomyces coelicolor and Bacillus subtilis and to SigB, a stress-response sigma factor, from B. subtilis. Southern blot surveys with a sigF-specific probe identified crosshybridizing bands in other slow-growing ***mycobacteria***, ***Mycobacterium*** bovis bacille Calmette-Guerin (BCG) and ***Mycobacterium*** avium, but not in the rapid-growers ***Mycobacterium*** smegmatis or ***Mycobacterium*** abscesses. RNase protection assays revealed that M. tuberculosis sigF mRNA is not present during exponential-phase growth in M. bovis BCG cultures but is strongly induced during stationary phase, nitrogen depletion, and cold shock. Weak expression of M. tuberculosis sigF was also detected during late-exponential phase, oxidative stress, anaerobiasis, and alcohol shock. The specific expression of M. tuberculosis sigF during stress or stationary phase suggests that it may play a role in the ability of tubercle bacilli to adapt to host defenses and persist during human infection.

L6 ANSWER 71 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1996:194438 CAPLUS
 DN 124:257720
 TI Immune responses to stress proteins in ***mycobacterial*** infections
 AU Ivanyi, Juraj; Norton, Pamela M.; Matsuzaki, Goro
 CS MRC Clinical Sciences Centre, Royal Postgraduate Medical School, London, UK
 SO Stress Proteins in Medicine (1996), 265-85. Editor(s): ***Van Eden,***
 *** Willem; Young, Douglas B***. Publisher: Dekker, New York, N. Y.
 CODEN: 62NSA5
 DT Conference; General Review
 LA English
 AB A review with 96 refs. on the immunogenicity of stress proteins during infections with pathogenic ***mycobacteria***. Topics discussed include hsp 65, hsp 10, hsp 71, .alpha.-crystallin, T-cell responses, antibody responses, and expression of host stress proteins.

L6 ANSWER 72 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1996:194434 CAPLUS
 DN 124:257718
 TI T-lymphocyte recognition of hsp 60 in experimental arthritis
 AU Anderton, Stephen M.; van Eden, Willem
 CS Institute Infectious Diseases and Immunology, University Utrecht, Utrecht, Neth.

SO Stress Proteins in Medicine (1996), 73-91. Editor(s): ***Van Eden,***
 *** Willem; Young, Douglas B*** . Publisher: Dekker, New York, N. Y.
 CODEN: 62NSA5

DT Conference; General Review
 LA English

AB A review, with 64 refs., discussing the first evidence for hsps as
 antigens in arthritis, responses to hsp 65 in adjuvant arthritis and other
 arthritis models, modulation of adjuvant arthritis using the
 arthritis-assocd. 180-188 epitope and peptide analogs, modulation of
 arthritis-assocd. using recombinant ***mycobacterial*** hsp 65, and
 T-cell reactivity to self hsp 60 as a protective mechanism in arthritis.

L6 ANSWER 73 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 AN 1996:496757 BIOSIS
 DN PREV199699219113
 TI The intracellular fate of ***mycobacteria*** in macrophages: Exclusion
 of BCG from the endosomal-lysosomal pathway.

AU Hasan, Zahra (1); Schlax, Claudia; ***Young, Douglas (1)*** ; Thole,
 Jelle (1); Pieters, Jean
 CS (1) Dep. Med. Microbiol., Imperial Coll. Sch. Med. St. Mary's, London UK
 SO Journal of Medical Microbiology, (1996) Vol. 45, No. 3, pp. I.
 Meeting Info.: 173rd Meeting of the Pathological Society of Great Britain
 and Ireland on the Molecular Basis of Intracellular Survival Southampton,
 England, UK July 10-12, 1996
 ISSN: 0022-2615.

DT Conference
 LA English

L6 ANSWER 74 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 AN 1996:871 BIOSIS
 DN PREV199698573006
 TI Prospects for new interventions in the treatment and prevention of
 mycobacterial disease.

AU ***Young, Douglas B. (1)*** ; Duncan, Kenneth
 CS (1) Dep. Med. Microbiol., St. Mary's Hosp. Med. Sch., Imperial Coll.,
 London W2 1PG UK
 SO Ornston, L. N. [Editor]. Annual Review of Microbiology, (1995) Vol. 49,
 pp. 641-673. Annual Review of Microbiology.
 Publisher: Annual Reviews Inc. P.O. Box 10139, 4139 El Camino Way, Palo
 Alto, California 94306, USA.
 ISSN: 0066-4227. ISBN: 0-8243-1149-3.

DT Book; General Review
 LA English

L6 ANSWER 75 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1995:881074 CAPLUS
 DN 123:282172
 TI Prospects for new interventions in the treatment and prevention of
 mycobacterial disease

AU ***Young, Douglas B.*** ; Duncan, Kenneth
 CS Department Medical Microbiology, St. Mary's Hospital Medical School,
 Imperial College, London, W2 1PG, UK
 SO Annual Review of Microbiology (1995), 49, 641-73
 CODEN: ARMIAZ; ISSN: 0066-4227

PB Annual Reviews
 DT Journal; General Review
 LA English

AB A review with 163 refs. ***Mycobacterium*** tuberculosis claims more lives each year than any other single human pathogen. Despite the availability of effective drugs, the incidence of tuberculosis is increasing in much of the developing world and has recently reemerged as a public health problem in industrialized countries. In the first section of this chapter, current understanding of the fundamental biol. of ***mycobacterial*** infection is reviewed from the perspective of development of new tools for disease control. A second section describes strategies for identification of novel antimycobacterial agents, with particular emphasis on recent progress in defining biosynthetic pathways for unique ***mycobacterial*** cell wall components. The third section focuses on current approaches to the development of new vaccine candidates consisting of live attenuated bacteria or individual antigenic subunits.

L6 ANSWER 76 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1995:147959 BIOSIS
DN PREV199598162259
TI Expression of heterologous genes in novel ***mycobacterial*** vectors.
AU O'Gaora, Peadar; Hayward, Chris; Thole, Jelle; ***Young, Douglas***
CS Dep. Med. Microbiol., St. Mary's Hosp. Med. Sch., Norfolk Place, London W2 1PG UK
SO Journal of Cellular Biochemistry Supplement, (1995) Vol. 0, No. 19B, pp. 77.
Meeting Info.: Keystone Symposium on Molecular Mechanisms in Tuberculosis
Tamarron, Colorado, USA February 19-25, 1995
ISSN: 0733-1959.
DT Conference
LA English

L6 ANSWER 77 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1995:147912 BIOSIS
DN PREV199598162212
TI Post-translational glycosylation of the ***Mycobacterium*** tuberculosis 19-kDa protein.
AU Abou-Zeid, Christiane (1); Zhang, Ying (1); ***Young, Douglas B. (1)***
; Dobos, Karen M.; Chatterjee, Delphi; Khoo, Kay-Hooi; Brennan, Patrick J.
CS (1) Dep. Med. Microbiol., St. Mary's Hosp. Med. Sch., London W2 1PG UK
SO Journal of Cellular Biochemistry Supplement, (1995) Vol. 0, No. 19B, pp. 65.
Meeting Info.: Keystone Symposium on Molecular Mechanisms in Tuberculosis
Tamarron, Colorado, USA February 19-25, 1995
ISSN: 0733-1959.
DT Conference
LA English

L6 ANSWER 78 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1995:147892 BIOSIS
DN PREV199598162192
TI M. tuberculosis proteins expressed in recombinant ***mycobacterial*** hosts.
AU ***Young, Douglas B. (1)*** ; Zhang, Ying (1); Cooper, Jon; Abou-Zeid, Christiane (1); Brennan, Patrick
CS (1) Dep. Med. Microbiol., St. Mary's Hosp. Med. Sch., London W2 1PG UK
SO Journal of Cellular Biochemistry Supplement, (1995) Vol. 0, No. 19B, pp. 57.
Meeting Info.: Keystone Symposium on Molecular Mechanisms in Tuberculosis

Tamarron, Colorado, USA February 19-25, 1995

ISSN: 0733-1959.

DT Conference
LA English

L6 ANSWER 79 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1994:414729 BIOSIS
DN PREV199497427729
TI Strategies for new drug development.
AU ***Young, Douglas B.***
CS Dep. Med. Microbiol., St. Mary's Hosp. Med. Sch., Norfolk Place, London W2
1PG UK
SO Bloom, B. R. [Editor]. (1994) pp. 559-567. Tuberculosis: Pathogenesis,
protection, and control.
Publisher: American Society for Microbiology (ASM) Books Division, 1325
Massachusetts Ave. NW, Washington, DC 20005-4171, USA.
ISBN: 1-55581-072-1.

DT Book
LA English

L6 ANSWER 80 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 40
AN 1994:253191 BIOSIS
DN PREV199497266191
TI Beating the bacillus.
AU ***Young, Douglas B.***
CS Dep. Med. Microbiol., St. Mary's Hosp. Med. Sch., Norfolk Pl., London W2
1PG UK
SO Current Biology, (1994) Vol. 4, No. 4, pp. 351-353.
ISSN: 0960-9822.

DT Article
LA English

L6 ANSWER 81 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 41
AN 1994:528649 BIOSIS
DN PREV199497541649
TI Molecular genetics of drug resistance in ***Mycobacterium***
tuberculosis.
AU Zhang, Ying; ***Young, Douglas***
CS Dep. Med. Microbiol., St. Mary's Hosp. Med. Sch., Imperial Coll. Sci.
Technol. Med., London W2 1PG UK
SO Journal of Antimicrobial Chemotherapy, (1994) Vol. 34, No. 3, pp. 313-319.
ISSN: 0305-7453.

DT General Review
LA English

AB Tuberculosis (TB) is the single largest killer among infectious diseases.
The recent resurgence of TB together with outbreaks of multidrug resistant
tuberculosis has focused attention on understanding the mechanisms of such
drug resistance. Because of the relative neglect of TB research in the
past and late arrival of ***mycobacterial*** genetic tools, the
molecular mechanisms of drug resistance in TB remained largely unknown
until very recently. In this paper we review recent progress on the
mechanisms of resistance to three major anti-TB drugs; isoniazid,
rifampicin and streptomycin. While the resistance mechanisms for
rifampicin and streptomycin are similar to those found in other bacteria,
isoniazid susceptibility and resistance is unique to ***Mycobacterium***

tuberculosis. So far, mutations in two chromosomal loci, katG and inhA have been found to be involved in isoniazid resistance in TB. Identification and characterization of mutations responsible for resistance opens up new possibilities for rapid detection of drug resistant strains. Molecular understanding of drug resistance and drug action in M. tuberculosis may eventually lead to rational design of new anti-TB drugs.

- L6 ANSWER 82 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 42
- AN 1994:545294 BIOSIS
- DN PREV199598004842
- TI Strain variation in the katG region of ***Mycobacterium***
tuberculosis.
- AU Zhang, Ying (1); ***Young, Douglas***
- CS (1) Dep. Med. Microbiol., St Mary's Hosp. Med. Sch., Norfolk Place, London
W2 1PG UK
- SO Molecular Microbiology, (1994) Vol. 14, No. 2, pp. 301-308.
ISSN: 0950-382X.
- DT Article
- LA English
- AB Southern blot analysis of chromosomal DNA from clinical isolates of
Mycobacterium tuberculosis using cosmid DNA probes revealed
extensive strain variation in the katG region of the genome. In addition
to deletion of the katG gene itself in some isoniazid-resistant strains,
adjacent DNA fragments were missing or altered in a range of
drug-sensitive and drug-resistant isolates. A species-specific 2 kb KpnI
fragment located 10 kb upstream of katG in M. tuberculosis H37Rv
hybridized to fragments of differing size in different clinical isolates
and was characterized in detail. Sequence analysis of this fragment showed
that it comprised three tandem copies of a novel 75 bp repeat element
flanked by multiple copies of the previously described 10 pb major
polymorphic tandem repeat of M. tuberculosis (MPTR). The copy number of
the 75 bp repeat was found to vary between strains, allowing application
of a polymerase chain reaction amplification strategy for strain
differentiation. These results indicate that the katG region of the M.
tuberculosis genome is highly variable and unstable. The presence of
repetitive sequences may contribute to instability in this region of the
genome.
- L6 ANSWER 83 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 43
- AN 1994:270395 BIOSIS
- DN PREV199497283395
- TI Transformation of ***mycobacterial*** species using hygromycin
resistance as selectable marker.
- AU Garbe, Thomas R.; Barathi, Jaya; Barnini, Simona; Zhang, Ying; Abou-Zeid,
Christiane; Tang, Dan; Mukherjee, Rama; ***Young, Douglas B. (1)***
- CS (1) Dep. Med. Microbiol., St. Mary's Hosp. Med. Sch., Norfolk Place,
London W2 1PG UK
- SO Microbiology (Reading), (1994) Vol. 140, No. 1, pp. 133-138.
- DT Article
- LA English
- AB Electroporation with shuttle plasmids carrying a kanamycin resistance gene
as a selectable marker failed to generate transformants in two
mycobacterial species currently being used in human vaccine
trials

(***Mycobacterium*** w and ***Mycobacterium*** vaccae). In contrast, efficient transformation (10⁻³-10⁻⁵ transformants (mu-g DNA)-1) was obtained using novel vectors with selection based on expression of resistance to hygromycin. The hygromycin resistance vector was also found to be more efficient than kanamycin resistance vectors for transformation of ***Mycobacterium*** smegmatis and ***Mycobacterium*** bovis BCG. The hygromycin resistance vector was used to overexpress superoxide dismutase of ***Mycobacterium*** tuberculosis in M. vaccae in a form suitable for detailed structural analysis. The potential use of this approach for generation of novel recombinant ***mycobacterial*** vaccines is discussed.

L6 ANSWER 84 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 44

AN 1994:306321 BIOSIS

DN PREV199497319321

TI Mapping of Hsp70-binding sites on protein antigens.

AU Roman, Eulogia (1); Moreno, Carlos; ***Young, Douglas***

CS (1) MRC Tuberculosis Related Infect. Unit, Royal Postgraduate Med. Sch.,
Hammersmith Hosp., London W12 OHS UK

SO European Journal of Biochemistry, (1994) Vol. 222, No. 1, pp. 65-73.
ISSN: 0014-2956.

DT Article

LA English

AB Hsp70-binding sites were mapped on three antigens, the 16-, 19- and 38-kDa proteins of ***Mycobacterium*** tuberculosis, using overlapping synthetic peptides in a competitive-binding assay. In each protein, two or three prominent hsp70-binding sites were identified when peptides 20-amino-acid long were used, predominantly in regions containing clusters of aliphatic amino acids. Although there was an overall concordance in the pattern of peptide binding to hsp70 from bacterial (M. tuberculosis) and mammalian sources (immunoglobulin heavy-chain-binding protein), some differences in the specificity of polypeptide binding and the effect of peptides on ATPase activity were observed.

L6 ANSWER 85 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1994:187176 CAPLUS

DN 120:187176

TI Rapid detection of antibiotic resistance in ***Mycobacterium***
tuberculosis

IN Heym, Beate; Cole, Stewart; ***Young, Douglas*** ; Zhang, Ying; Honore,
Nadine; Telenti, Amalio; Bodmer, Thomas

PA Institut Pasteur, Fr.; Medical Research Council; Assistance Publique;
Universite Pierre et Marie Curie; Universite de Berne

SO PCT Int. Appl., 96 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9322454	A1	19931111	WO 1993-EP1063	19930430
	W: CA, JP, US				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	FR 2695650	A1	19940318	FR 1992-11098	19920917
	FR 2695650	B1	19941125		
	JP 07059595	A2	19950307	JP 1992-312596	19921009

JP 3408564	B2	20030519		
JP 2003225097	A2	20030812	JP 2002-374077	19921009
FR 2704002	A1	19941021	FR 1993-4545	19930416
FR 2704002	B1	19950707		
EP 639229	A1	19950222	EP 1993-909875	19930430
EP 639229	B1	19990310		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 07506003	T2	19950706	JP 1993-518923	19930430
AT 177476	E	19990315	AT 1993-909875	19930430
ES 2131578	T3	19990801	ES 1993-909875	19930430
US 5851763	A	19981222	US 1994-313185	19941012
US 6124098	A	20000926	US 1998-82614	19980520
PRAI US 1992-875940	A	19920430		
US 1992-929206	A	19920814		
FR 1992-11098	A	19920917		
FR 1993-4545	A	19930416		
JP 1992-312596	A3	19921009		
WO 1993-EP1063	W	19930430		
AB	<p>Multidrug resistant strains of ***Mycobacterium*** tuberculosis represent a considerable threat to public health worldwide. Resistance to isoniazid (INH), rifampicin or analogs thereof, of streptomycin, i.e. key components of anti-tuberculosis regimens, need frequently to be detected. The invention involves the detection of a mutation in either the katG gene (isoniazid resistance), the rpoB gene (rifampicin resistance) or rpsL gene (streptomycin resistance). Cloning of genes katG, rpoB, and rpsL was demonstrated and their sequences disclosed.</p>			
L6	ANSWER 86 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 45			
AN	1993:387581 BIOSIS			
DN	PREV199396062881			
TI	Characterization of the katG gene encoding a catalase-peroxidase required for the isoniazid susceptibility of ***Mycobacterium*** tuberculosis.			
AU	Heym, Beate; Zhang, Ying; Poulet, Sylvie; ***Young, Douglas*** ; Cole, Stewart T. (1)			
CS	(1) Unite de Genetique Mol. Bacterienne, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15 France			
SO	Journal of Bacteriology, (1993) Vol. 175, No. 13, pp. 4255-4259. ISSN: 0021-9193.			
DT	Article			
LA	English			
AB	<p>The isoniazid susceptibility of ***Mycobacterium*** tuberculosis is mediated by the product of the katG gene which encodes the heme-containing enzyme catalase-peroxidase. In this study, the chromosomal location of katG has been established and its nucleotide sequence has been determined so that the primary structure of catalase-peroxidase could be predicted. The M. tuberculosis enzyme is an 80,000-dalton protein containing several motifs characteristic of peroxidases and shows strong similarity to other bacterial catalase-peroxidases. Expression of the katG gene in M. tuberculosis, M. smegmatis, and Escherichia coli was demonstrated by Western blotting (immunoblotting). Homologous genes were detected in other ***mycobacteria***, even those which are naturally insensitive to isoniazid.</p>			
L6	ANSWER 87 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 46			
AN	1993:342481 BIOSIS			

DN PREV199396039481
 TI Transformation with katG restores isoniazid sensitivity in
 Mycobacterium tuberculosis isolates resistant to a range of drug
 concentrations.
 AU Zhang, Ying; Garbe, Thomas; ***Young, Douglas (1)***
 CS (1) MRC Tuberculosis Related Infections Unit, Hammersmith Hospital, Ducane
 Road, London W12 0HS UK
 SO Molecular Microbiology, (1993) Vol. 8, No. 3, pp. 521-524.
 ISSN: 0950-382X.
 DT Article
 LA English
 AB Isoniazid-resistant isolates of ***Mycobacterium*** tuberculosis were
 transformed with a plasmid vector carrying the functional
 catalase-peroxidase (katG) gene. Expression of katG restored full drug
 susceptibility in isolates initially resistant to concentrations ranging
 from 3.2 to gt 50 mu-g ml-1. Transformation with the corresponding katG
 gene from Escherichia coli resulted in low-level expression of catalase
 and peroxidase activities and conferred partial isoniazid sensitivity.

L6 ANSWER 88 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 47
 AN 1993:141371 BIOSIS
 DN PREV199395074171
 TI Expression of the ***Mycobacterium*** tuberculosis 19-Kilodalton
 antigen in ***Mycobacterium*** smegmatis: Immunological analysis and
 evidence of glycosylation.
 AU Garbe, Thomas (1); Harris, David; Vordermeier, Martin; Lathigra, Raju;
 Ivanyi, Juraj; ***Young, Douglas***
 CS (1) Dep. Mol. Genetics, Biochem. Microbiol., University Cincinnati College
 Med., 3110 Med. Sci. Building, 231 Bethesda Ave., Cincinnati, Ohio
 45267-0524
 SO Infection and Immunity, (1993) Vol. 61, No. 1, pp. 260-267.
 ISSN: 0019-9567.
 DT Article
 LA English
 AB The gene encoding a 19-kDa antigen from ***Mycobacterium***
 tuberculosis was expressed as a recombinant protein in the rapid-growing
 species ***Mycobacterium*** smegmatis. The recombinant antigen was
 expressed at a level approximately ninefold higher than in M. tuberculosis
 and, like the native antigen, was found in the pellet fraction after
 high-speed centrifugation of bacterial extracts. The 19-kDa antigen in
 crude bacterial extracts, and the purified recombinant antigen, bound
 strongly to concanavalin A, indicating the possibility of
 posttranslational glycosylation. The recombinant antigen stimulated T-cell
 proliferation in vitro when added to assays either in the form of whole
 recombinant bacteria or as a purified protein. Homologous expression of
 mycobacterial antigens in a rapid-growing ***mycobacterial***
 host may be particularly useful for the immunological characterization of
 proteins which are subject to posttranslational modification.

L6 ANSWER 89 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 48
 AN 1993:366128 BIOSIS
 DN PREV199396051803
 TI Protective immunity elicited by recombinant bacille Calmette-Guerin (BCG)
 expressing outer surface protein A (OspA) lipoprotein: A candidate Lyme
 disease vaccine.

AU Stover, C. Kendall (1); Bansal, Geetha P.; Hanson, Mark S.; Burlein, Jeanne E.; Palaszynski, Susan R.; Young, James F.; Koenig, Scott; ***Young, Douglas B.*** ; Sadziene, Ariadna; Barbour, Alan G.

CS (1) Dep. Mol. Microbiol., MedImmune Inc., 35 West Watkins Mill Rd., Gaithersburg, MD 20878 USA

SO Journal of Experimental Medicine, (1993) Vol. 178, No. 1, pp. 197-209. ISSN: 0022-1007.

DT Article

LA English

AB The current vaccine against tuberculosis, ***Mycobacterium*** bovis strain bacille Calmette-Guerin (BCG), offers potential advantages as a live, innately immunogenic vaccine vehicle for the expression and delivery of protective recombinant antigens (Stover, C. K., V. F. de la Cruz, T. R. Fuerst, J. E. Burlein, L. A. Benson, L. T. Bennett, G. P. Bansal, J. F. Young, M. H. Lee, G. F. Hatfull et al. 1991. Nature (Lond.) 351:456; Jacobs, W. R., Jr., S. B. Snapper, L. Lugosi and B. R. Bloom. 1990. Curr. Top. Microbiol. Immunol. 155:153; Jacobs, W. R., M. Tuckman, and B. R. Bloom. 1987. Nature (Lond.). 327:532); but as an attenuated intracellular bacterium residing in macrophages, BCG would seem to be best suited for eliciting cellular responses and not humoral responses. Since bacterial lipoproteins are often among the most immunogenic of bacterial antigens, we tested whether BCG expression of a target antigen as a membrane-associated lipoprotein could enhance the potential for a recombinant BCG vaccine to elicit high-titered protective antibody responses to target antigens. Immunization of mice with recombinant BCG vaccines expressing the outer surface protein A (OspA) antigen of *Borrelia burgdorferi* as a membrane-associated lipoprotein resulted in protective antibody responses that were 100-1,000-fold higher than responses elicited by immunization with recombinant BCG expressing OspA cytoplasmically or as a secreted fusion protein. Furthermore, these improved antibody responses were observed in heterogeneous mouse strains that vary in their immune responsiveness to OspA and sensitivity to BCG growth. Thus, expression of protective antigens as chimeric membrane-associated lipoproteins on recombinant BCG may result in the generation of new candidate vaccines against Lyme borreliosis and other human or veterinary diseases where humoral immunity is the protective response.

L6 ANSWER 90 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

AN 1994:296818 BIOSIS

DN PREV199497309818

TI Molecular mechanisms of isoniazid: A drug at the front of tuberculosis control.

AU Zhang, Ying; ***Young, Douglas B.***

CS MRC Tuberculosis and Related Infections Unit, RPMS, Hammersmith Hosp., Ducane Road, London W12 0HS UK

SO Trends in Microbiology, (1993) Vol. 1, No. 3, pp. 109-113. ISSN: 0966-842X.

DT General Review

LA English

L6 ANSWER 91 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

AN 1994:417180 BIOSIS

DN PREV199497430180

TI ***Mycobacterium*** leprae infection triggers synthesis of stress inducible hsp 70 in Schwann cells and anti hsp 70 antibodies in sera.

AU Misry, Yasin (1); ***Young, Douglas B.*** ; Mukherjee, Rama (1)

CS (1) Natl. Inst. Immunol., Shahid Jeet Singh Marg, New Delhi-110067 India

SO International Journal of Leprosy and Other Mycobacterial Diseases, (1993)
Vol. 61, No. 4 SUPPL., pp. 104A-105A.
Meeting Info.: Fourteenth International Leprosy Congress Orlando, Florida,
USA August 29-September 4, 1993
ISSN: 0148-916X.

DT Conference
LA English

L6 ANSWER 92 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1994:417088 BIOSIS
DN PREV199497430088
TI Heat shock proteins in leprosy reversal reactions.
AU Lockwood, Diana (1); ***Young, Douglas*** ; Colston, Jo; Stanley, John;
Young, Saroj (1)
CS (1) Dep. Clinical Sci., London Sch. Hygiene Tropical Med., London WC1E 7HT
UK

SO International Journal of Leprosy and Other Mycobacterial Diseases, (1993)
Vol. 61, No. 4 SUPPL., pp. 84A.
Meeting Info.: Fourteenth International Leprosy Congress Orlando, Florida,
USA August 29-September 4, 1993
ISSN: 0148-916X.

DT Conference
LA English

L6 ANSWER 93 OF 121 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 49
AN 1993:126425 BIOSIS
DN PREV199344057425
TI Leprosy, tuberculosis, and the new genetics.
AU ***Young, Douglas B. (1)*** ; Cole, Stewart T.
CS (1) MRC Tuberculosis Related Infections Unit, Hammersmith Hosp., Ducane
Rd., London W12 0HS UK

SO Journal of Bacteriology, (1993) Vol. 175, No. 1, pp. 1-6.
ISSN: 0021-9193.

DT General Review
LA English

L6 ANSWER 94 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1992:589315 CAPLUS
DN 117:189315
TI Hsp70 Synthesis in Schwann cells in response to heat shock and infection
with ***Mycobacterium*** leprae
AU Mistry, Yasmin; ***Young, Douglas B.*** ; Mukherjee, Rama
CS Microbiol. Div., Natl. Inst. Immunol., New Delhi, 110 067, India
SO Infection and Immunity (1992), 60(8), 3105-10
CODEN: INFIBR; ISSN: 0019-9567

DT Journal
LA English
AB Induction of heat shock protein synthesis was monitored in murine and
monkey Schwann cells exposed to elevated temps. Synthesis of the
stress-inducible 70-kDa heat shock protein (hsp70) was detected in both
murine and primate Schwann cells by metabolic labeling and by
immunoblotting with a specific monoclonal antibody. Hsp70 synthesis was
also induced in Schwann cells after infection with ***Mycobacterium***
leprae and was detected from 24 h to 1 wk postinfection. These results
are discussed with respect to the possible role of heat shock proteins in
immunopathol. events assocd. with the clin. manifestations of leprosy.

L6 ANSWER 95 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1993:166887 CAPLUS
 DN 118:166887
 TI The 14,000-molecular-weight antigen of ***Mycobacterium***
 tuberculosis is related to the alpha-crystallin family of
 low-molecular-weight heat shock proteins
 AU Verbon, Annelies; Hartskeerl, Rudy A.; Schuitema, Anja; Kolk, Arend H. J.;
 Young, Douglas B. ; Lathigra, Ruju
 CS N. H. Swellengrebel Inst. Trop. Hyg., R. Trop. Inst., Amsterdam, Neth.
 SO Journal of Bacteriology (1992), 174(4), 1352-9
 CODEN: JOBAAY; ISSN: 0021-9193
 DT Journal
 LA English
 AB Eight monoclonal antibodies (MAbs) directed against the 14,000-mol.-wt.
 (14K) antigen of M. tuberculosis reacted specifically with
 mycobacteria of the M. tuberculosis complex. The nucleotide
 sequence of the gene encoding the 14K antigen was detd. by using
 recombinant DNA clones isolated from lambda gt11 and cosmid libraries of
 the M. tuberculosis genome. The DNA sequence of the 14K protein gene
 coded for a polypeptide of 144 amino acids with a calcd. mol. mass of
 16,277 Da. The 14K antigen has a marked homol. with proteins belonging to
 the .alpha.-crystallin family of low-mol.-wt. heat shock proteins, which
 includes the 18K antigen of M. leprae. The 8 MAbs recognized at least 4
 distinct epitopes localized within the following 3 regions of the 14K
 protein: amino acids 10-92 (MAbs F67-8 and F67-16), amino acids 41-92
 (F159-1 and F159-11), and amino acids 41-144 (F23-41, F24-2, F23-49, and
 TB68).

L6 ANSWER 96 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1992:528055 CAPLUS
 DN 117:128055
 TI The catalase-peroxidase gene and isoniazid resistance of
 Mycobacterium tuberculosis
 AU Zhang, Ying; Heym, Beate; Allen, Bryan; ***Young, Douglas*** ; Cole,
 Stewart
 CS R. Postgrad. Med. Sch., Hammersmith Hosp., London, W12 0HS, UK
 SO Nature (London, United Kingdom) (1992), 358(6387), 591-3
 CODEN: NATUAS; ISSN: 0028-0836
 DT Journal
 LA English
 AB Tuberculosis is responsible for 1 in 4 of all avoidable adult deaths in
 developing countries. Increased frequency and accelerated fatality of the
 disease among individuals infected with human immunodeficiency virus has
 raised worldwide concern that control programs may be inadequate, and the
 emergence of multidrug-resistant strains of M. tuberculosis has resulted
 in several recent fatal outbreaks in the United States. Isonicotinic acid
 hydrazid (isoniazid, INH) forms the core of antituberculosis regimens;
 however, clin. isolates that are resistant to INH show reduced catalase
 activity and a relative lack of virulence in guinea pigs.
 Mycobacterial genetics were used to study the mol. basis of INH
 resistance. A single M. tuberculosis gene, katG, encoding both catalase
 and peroxidase, restored sensitivity to INH in a resistant mutant of
 Mycobacterium smegmatis, and conferred INH susceptibility in some
 strains of Escherichia coli. Deletion of katG from the chromosome was
 assocd. with INH resistance in 2 patient isolates of M. tuberculosis.

L6 ANSWER 97 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1991:578996 CAPLUS
 DN 115:178996
 TI Heat shock proteins and antigens of ***Mycobacterium*** tuberculosis
 AU ***Young, Douglas B.*** ; Garbe, Thomas R.
 CS Med. Res. Counc. Tuberc. Relat. Infect. Unit, Hammersmith Hosp., London, W12 0HS, UK
 SO Infection and Immunity (1991), 59(9), 3086-93
 CODEN: INFIBR; ISSN: 0019-9567
 DT Journal
 LA English
 AB The heat shock response of ***Mycobacterium*** tuberculosis has been characterized in detail by one- and two-dimensional PAGE after metabolic labeling with [35S]methionine and 14C-amino acids. A temp. increase from 37 to 42.degree. induced elevated synthesis of 3 major proteins corresponding to the DnaK, GroEL, and GroES proteins of M. tuberculosis previously identified as prominent antigens. At higher temps. (45 to 48.degree.), synthesis of GroEL decreased and novel heat shock proteins with mol. masses of 90, 28, 20, and 15 kDa were obsd. These new proteins did not comigrate with known antigens during two-dimensional gel electrophoresis. The heat shock response is discussed with regard to the possible importance of transcriptional regulation of ***mycobacterial*** genes in vivo.

L6 ANSWER 98 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1991:599841 CAPLUS
 DN 115:199841
 TI Genetic and immunological analysis of ***Mycobacterium*** tuberculosis fibronectin-binding proteins
 AU Abou-Zeid, Christiane; Garbe, Thomas; Lathigra, Raju; Wiker, Harald G.; Harboe, Morten; Rook, Graham A. W.; ***Young, Douglas B.***
 CS Middlesex Sch. Med., Univ. Coll., London, W1P 7PN, UK
 SO Infection and Immunity (1991), 59(8), 2712-18
 CODEN: INFIBR; ISSN: 0019-9567
 DT Journal
 LA English
 AB Recombinant phage clones, TB1 and TB2, were selected from a M. tuberculosis .lambda.gt11 DNA expression library by screening with a polyclonal antiserum raised against the antigen 85 complex of ***Mycobacterium*** bovis BCG. Anal. of recombinant DNA inserts and expressed fusion proteins showed that two new genes had been isolated. The product of clone TB2 was identified as a member of the 30/31-kDa antigen 85 complex. Restriction enzyme anal. showed that this gene differs from previously cloned members of this antigen complex, with detailed serol. anal. indicating that it may encode the 85C component. Antisera raised against the expressed product of clone TB1 recognized a 55-kDa protein in M. tuberculosis exts. The 55-kDa protein also has fibronectin-binding activity and, like the 30/31-kDa family, is a prominent target of the antibody response in patients with ***mycobacterial*** disease. Although the clones were selected by using the same antiserum, detailed anal. by serol. and by DNA hybridization showed that they represent two quite distinct types of fibronectin-binding activities expressed by M. tuberculosis. Further anal. of the fibronectin-binding antigens in M. tuberculosis may provide important insights into their role in mediating the interaction with the host immune system.

L6 ANSWER 99 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1992:627292 CAPLUS
 DN 117:227292
 TI The ***Mycobacterium*** tuberculosis shikimate pathway genes:
 evolutionary relationship between biosynthetic and catabolic
 3-dehydroquinases
 AU Garbe, Thomas; Servos, Spiros; Hawkins, Alastair; Dimitriadis, George;
 Young, Douglas ; Dougan, Gordon; Charles, Ian
 CS MRC Tuberc. Relat. Infect. Unit, RPMS, London, W12 0HS, UK
 SO Molecular and General Genetics (1991), 228(3), 385-92
 CODEN: MGGEAE; ISSN: 0026-8925
 DT Journal
 LA English
 AB The M. tuberculosis shikimate pathway genes designated aroB and aroQ
 encoding 3-dehydroquinate synthase and 3-dehydroquinase, resp. were
 isolated by mol. cloning and their nucleotide sequences detd. The deduced
 dehydroquinate synthase amino acid sequence from M. tuberculosis showed
 high similarity to those of equiv. enzymes from prokaryotes and
 filamentous fungi. Surprisingly, the deduced M. tuberculosis
 3-dehydroquinase amino acid sequence showed no similarity to other
 characterized prokaryotic biosynthetic 3-dehydroquinases (bdHQases). A
 high degree of similarity was obsd., however, to the fungal catabolic
 3-dehydroquinases (cdHQases) which are active in the quinic acid
 utilization pathway and are isoenzymes of the fungal bdHQases. This
 finding indicates a common ancestral origin for genes encoding the
 catabolic dehydroquinases of fungi and the biosynthetic dehydroquinases
 present in some prokaryotes. Deletion of genes encoding shikimate pathway
 enzymes represents a possible approach to generation of rationally
 attenuated strains of M. tuberculosis for use as live vaccines.

L6 ANSWER 100 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1991:222288 CAPLUS
 DN 114:222288
 TI Cloning and characterization of the aroA gene from ***Mycobacterium***
 tuberculosis
 AU Garbe, Thomas; Jones, Christopher; Charles, Ian; Dougan, Gordon;
 Young, Douglas
 CS R. Postgrad. Med. Sch., Hammersmith Hosp., London, W12 0HS, UK
 SO Journal of Bacteriology (1990), 172(12), 6774-82
 CODEN: JOBAAY; ISSN: 0021-9193
 DT Journal
 LA English
 AB The aroA gene from M. tuberculosis was cloned by complementation of an
 aroA mutant of Escherichia coli after lysogenization with a recombinant
 DNA library in the .lambda.gt11 vector. Detailed characterization of the
 M. tuberculosis aroA gene by nucleotide sequencing and by immunochem.
 anal. of the expressed product indicates that it encodes a
 5-enolpyruvylshikimate-3-phosphate synthase that is structurally related
 to analogous enzymes from other bacterial, fungal, and plant sources. The
 potential use of the cloned gene in construction of genetically defined
 mutant strains of M. tuberculosis by gene replacement is proposed as a
 novel approach to the rational attenuation of ***mycobacterial***
 pathogens and the possible development of new antimycobacterial vaccines.

L6 ANSWER 101 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1990:476112 CAPLUS

DN 113:76112
 TI Recognition of a peptide antigen by heat shock-reactive .gamma..delta. T lymphocytes
 AU Born, Willi; Hall, Lisa; Dallas, Angela; Boymel, Joel; Shinnick, Thomas; ***Young, Douglas*** ; Brennan, Patrick; O'Brien, Rebecca
 CS Health Sci. Cent., Univ. Colorado, Denver, CO, 80206, USA
 SO Science (Washington, DC, United States) (1990), 249(4964), 67-9
 CODEN: SCIEAS; ISSN: 0036-8075
 DT Journal
 LA English
 AB Small synthetic peptides that correspond to different portions of the 65-kilodalton ***mycobacterial*** heat shock protein (Hsp65) were used to identify a putative antigenic epitope for .gamma..delta. cells. Weaker .gamma..delta. responses to the equiv. portion of the autologous homolog, mouse Hsp63, were also seen. The stimulatory epitope overlaps with an epitope recognized by arthritogenic .alpha..beta. T cell clones. The data suggest that .gamma..delta. cells have a role in autoimmune disorders and imply that these cells recognize ligands by a mechanism similar to that of .alpha..beta. T lymphocytes, i.e., in the form of small processed protein fragments bound to antigen-presenting mols.

L6 ANSWER 102 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1990:530587 CAPLUS
 DN 113:130587
 TI Stress proteins and uses therefor
 IN Young, Richard A.; ***Young, Douglas***
 PA Whitehead Institute for Biomedical Research, USA; Medical Research Council
 SO PCT Int. Appl., 42 pp.
 CODEN: PIXXD2

DT Patent
 LA English

FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 8912455	A1	19891228	WO 1989-US2619	19890615
	W: JP				
	RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
	EP 419569	A1	19910403	EP 1989-907594	19890615
	EP 419569	B1	19950906		
	R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
	CA 1338778	A1	19961210	CA 1989-602924	19890615
	US 6338952	B1	20020115	US 1994-336251	19941103
	US 6335183	B1	20020101	US 1995-461722	19950605
	US 6482614	B1	20021119	US 1999-468041	19991221
	US 2003073094	A1	20030417	US 2002-46649	20020114
PRAI	US 1988-207298	A	19880615		
	US 1989-366581	B1	19890615		
	WO 1989-US2619	W	19890615		
	US 1991-804632	B2	19911209		
	US 1993-73381	B2	19930604		
	WO 1994-US6362	A2	19940606		
	US 1994-336251	B1	19941103		
	US 1995-461720	B1	19950605		
AB	Stress proteins, or all or a portion of a protein having an amino acid sequence sufficiently homologous to those of the stress proteins, are provided for use as vaccines. The stress proteins are also useful for induction of immune tolerance and treatment of autoimmune diseases, e.g.				

rheumatoid arthritis. Thus, DNA clones coding for ***Mycobacterium*** tuberculosis and M. lepral protein antigens are sequenced. For example, DNA encoding the M. tuberculosis 19 kilodalton (kDa) antigen and the M. leprae 18 kDa antigen (to which human T-cells are responsive) was sequenced. The M. tuberculosis 19 kDa protein exhibited no significant sequence similarity to proteins in the GenBank database, but the M. leprae 18 kDa protein sequence was similar to the soybean 17 kDa heat-shock protein. Homologies between protein antigens of M. tuberculosis/M. leprae and Escherichia coli DnaK and GroEL gene products are described and tabulated.

L6 ANSWER 103 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1989:210357 CAPLUS

DN 110:210357

TI Identification of ***mycobacterial*** antigens recognized by T lymphocytes

AU Lamb, Jonathan R.; Lathigra, Raju; Rothbard, Jonathan B.; Sweetser, Douglas; Young, Richard A.; Ivanyi, Juraj; ***Young, Douglas B.***

CS R. Postgrad. Med. Sch., Hammersmith Hosp., London, UK

SO Reviews of Infectious Diseases (1989), 11(Suppl. 2), S443-S447
CODEN: RINDDG; ISSN: 0162-0886

DT Journal; General Review

LA English

AB A review with 19 refs. Various approaches designed to analyze the recognition of ***mycobacterial*** antigens by T cells are reviewed. In addn. to the established approach of using serol. defined antigens, alternative methods independent of antibody preselection, such as polyacrylamide gel electrophoresis-fractionated immunoblots of ***mycobacteria***, can be used to probe the specificity of the T cell repertoire. Furthermore, the application of recombinant DNA expression combined with that of synthetic peptides whose sequences are predicted to constitute T cell determinants allow the localization of T cell epitopes within a protein.

L6 ANSWER 104 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1989:190595 CAPLUS

DN 110:190595

TI Orientation of epitopes influences the immunogenicity of synthetic peptide dimers

AU Cox, Josephine H.; Ivanyi, Juraj; ***Young, Douglas B.***; Lamb, Jonathan R.; Syred, Andrew D.; Francis, Michael J.

CS R. Postgrad. Med. Sch., Hammersmith Hosp., London, UK

SO European Journal of Immunology (1988), 18(12), 2015-19
CODEN: EJIMAF; ISSN: 0014-2980

DT Journal

LA English

AB The immunogenicity of synthetic peptide dimers based on epitope sequences derived from the ***mycobacterial*** 65-kDa antigen and the foot and mouth disease virus (FMDV) VP1 protein was examd. in inbred mice. The anal. was directed towards the potential helper role of a T cell stimulatory ***mycobacterial*** epitope (65-85) with respect to poorly immunogenic sites either from the same mol. (422-436) or from VP1 (141-160). The 65-85 repeat homodimer induced an antibody response in CBA/ca but not in C57BL/6 mice, both nonresponders to the 65-85 monomer, and amplified the antibody response in BALB/c, monomer-responder mice. Anal. of the immunogenicity of hybrid dimers in BALB/c mice showed that the orientation of peptides within the dimer is crit. for the extent of

the produced antibody response. Only the 422-436/65-85 but not the 65-85/422-436 induced antibodies binding to the 422-436 sequence which was nonimmunogenic when injected either as a monomer or dimer. Despite the striking difference in immunogenicity, both tested hybrid dimers reacted equally in the solid-phase immunoassay with antisera raised to 65-85-dimer or 422-436/65-85 peptides or with a monoclonal antibody to the 422-436 epitope. The described differences in antibody responsiveness also cannot be attributed merely to the extent of T cell stimulation since the proliferative responses were uniformly expressed for all relevant combinations of peptides. Antisera to 65-85 dimer and 422-436/65-85 hybrid also reacted with the native 65-kDa protein. Furthermore, the prodn. of FMDV-neutralizing antibodies in response to the 141-160 (VP1-derived)/65-85 hybrid peptide in 141-160 nonresponder B10.D2 mice also confirmed the helper activity of the 65-85 epitope. Thus, combining heterologous peptides with the N-terminal of the ***mycobacterial*** 65-85 sequence may be generally applicable for the potentiation of peptide vaccines.

L6 ANSWER 105 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1989:112768 CAPLUS
 DN 110:112768
 TI Specificity of proliferative response of human CD8 clones to
 mycobacterial antigens
 AU Rees, Ann; Scoging, Anne; Mehlert, Angela; ***Young, Douglas B.*** ;
 Ivanyi, Juraj
 CS R. Postgrad. Med. Sch., Hammersmith Hosp., London, W12 0HS, UK
 SO European Journal of Immunology (1988), 18(12), 1881-7
 CODEN: EJIMAF; ISSN: 0014-2980
 DT Journal
 LA English
 AB Human CD8 T lymphocyte clones (TLC) were generated from the pleural effusion of patients with tuberculosis using a protocol that required, in addn. to antigen, coculture of purified CD8+ T cells, accessory cells, interleukin 2 (IL 2) and anti-CD3-Sepharose. The TLC obtained were stimulated by ***mycobacterial*** sol. exts. in an IL 2-dependent and MHC class I-restricted manner. When antigen-responsive TLC were screened with exts. from the recombinant ***mycobacterial*** library they responded to either the Y3125 (100-kDa) or the Y3111 (71-kDa) .lambda.gt11 clones. Polyacrylamide gel immunoblot anal. demonstrated that the CD8 TLC responded to fractions with the mol. mass range 27-45 kDa in the Y3125 lysogen and 60-90 kDa in the ***mycobacterial*** sol. ext. These TLC recognized sequences common to the 71-kDa protein derived from ***mycobacteria***, E. coli or a human cell line. Stimulation with both the Y3125 and the 71-kDa antigens were restricted by determinants encoded by HLA-B8.

L6 ANSWER 106 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1988:432884 CAPLUS
 DN 109:32884
 TI A gene from ***Mycobacterium*** tuberculosis which is homologous to the DnaJ heat shock protein of E. coli
 AU Lathigra, Raju B.; ***Young, Douglas B.*** ; Sweetser, Doug; Young, Richard A.
 CS MRC Tuberc. Related Infect. Unit, Hammersmith Hosp., London, W12 0HS, UK
 SO Nucleic Acids Research (1988), 16(4), 1636
 CODEN: NARHAD; ISSN: 0305-1048
 DT Journal

LA English

AB Sequence anal. of an open reading frame located 788 base pairs downstream from the dnaK gene of *M. tuberculosis* indicates the occurrence of a 356 amino acid protein sharing considerable sequence homol. with the *Escherichia coli* gene dnaJ protein, including conservation of 4 tandem repeats of a motif consisting of Cys-X-X-Cys-X-Gly-X-Gly.

L6 ANSWER 107 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1988:202917 CAPLUS

DN 108:202917

TI Generation and characterization of monoclonal antibodies to 28-, 35-, and 65-kilodalton proteins of ****Mycobacterium**** *tuberculosis*

AU Damiani, Guido; Bianco, Annalisa; Beltrame, Anna; Vismara, Daniela; Filippone Mezzopreti, Marina; Colizzi, Vittorio; ***Young, Douglas B.***; Bloom, Barry R.

CS Inst. Biol. Chem., Univ. Genoa, Genoa, 16132, Italy

SO *Infection and Immunity* (1988), 56(5), 1281-7
CODEN: INFIBR; ISSN: 0019-9567

DT Journal

LA English

AB Three monoclonal antibodies (H60.15, H61.3, and H105.10) directed to protein antigens of *M. tuberculosis* were obtained and characterized. H60.15 recognizes a protein with a mol. mass of 28 kilodaltons (kDa) with broad cross-reactivity on a panel of 12 species and strains of ****mycobacteria****. H61.3 reacts with a 35-kDa protein present in *M. tuberculosis*, *M. bovis* BCG, and *M. africanum*. On the basis of the antigen mol. masses and competition expts. with other monoclonal antibodies, H60.15 and H61.3 seem to be the first described monoclonal antibodies to these *M. tuberculosis* proteins. H105.10 binds to the cross-reactive 65-kDa protein present in ****mycobacteria****. Epitope mapping of H105.10 was performed by using the *M. leprae* DNA sublibrary available in bacteriophage .lambda.gt11 for this antigen and revealed that its epitope resides in the region from amino acids 20 to 54. The 28-, 35-, and 65-kDa antigens isolated by immunoblotting and presented on nitrocellulose to pleural effusion T cells from tuberculosis patients induced a proliferative response, indicating the presence of T-cell epitopes. Thus, 2 protein antigens should be added to the list of antigens detectable in *M. tuberculosis* by monoclonal antibodies. The common feature of such proteins, the elicitation of an immune response of limited or broad cross-reactivity for ****mycobacteria****, encourages the search for their role in the pathogenesis of ****mycobacterioses****.

L6 ANSWER 108 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1988:202866 CAPLUS

DN 108:202866

TI Biological activity of protein antigens isolated from ****Mycobacterium**** *tuberculosis* culture filtrate

AU Collins, Frank M.; Lamb, Jonathan R.; ***Young, Douglas B.***

CS Med. Res. Counc. Tuberc. Relat. Dis. Uni, Hammersmith Hosp., London, W12 0HS, UK

SO *Infection and Immunity* (1988), 56(5), 1260-6
CODEN: INFIBR; ISSN: 0019-9567

DT Journal

LA English

AB *M. tuberculosis* Culture filtrate (MTCF) protein antigens were isolated from mid-logarithmic-phase cultures grown in liq. medium and examd. by HPLC and Western blot (immunoblot) anal. A major protein band with a mol.

mass of about 68 kilodaltons (kDa) and several fainter bands in the 38- and 24-kDa range were obsd. The MTCF protein produced a delayed footpad hypersensitivity response in *M. bovis* BCG-vaccinated C57BL/6 mice, comparable to that obsd. with PPD. The same proteins induced a blastogenic response in tuberculin-sensitive human peripheral blood monocytes and in T-cell clones developed from these cells. The proliferative responses to the MTCF antigens were equiv. to those obsd. following stimulation with PPD or *M. tuberculosis* sonic exts. However, the MTCF sensitins were not recognized by 5 monoclonal antibodies directed against killed *M. tuberculosis* antigens in an enzyme immunoassay, although some response was seen with a monoclonal antibody (ML34) directed against *M. leprae* antigens. The ability of the MTCF to stimulate T-cell responses both in vivo and in vitro while not being recognized by antibodies directed against dead ***mycobacterial*** antigens suggests that they may be of interest as potential protective immunogens.

L6 ANSWER 109 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1988:471808 CAPLUS

DN 109:71808

TI Analysis of stress-related proteins involved in the immune response to ***mycobacterial*** infection

AU Mehlert, Angela; Lamb, Jonathan; ***Young, Douglas***

CS R. Postgrad. Med. Sch., Hammersmith Hosp., London, W12 0HS, UK

SO Biochemical Society Transactions (1988), 16(5), 721-2

CODEN: BCSTB5; ISSN: 0300-5127

DT Journal

LA English

AB Two stress-related ***mycobacterial*** proteins were identified. A 65-kilodalton (kDa) protein was identified in ***Mycobacterium*** *bovis* which was immunol. cross-reactive with the GroEL protein of *Escherichia coli* and with a heat-shocked human lymphocyte ext. A 71-kDa protein was identified in *M. tuberculosis* which was immunol. cross-reactive with the dnaK gene product of *E. coli* and with the human lymphocyte hsp 70 protein. The role of these 2 stress-related ***mycobacterial*** proteins in the immune response to ***mycobacterial*** infections and in autoimmune diseases is briefly discussed.

L6 ANSWER 110 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1988:184720 CAPLUS

DN 108:184720

TI Immunoblotting and the immune response to leprosy

AU ***Young, Douglas B.***

CS MRC Tuberc. Related Infect. Unit, Hammersmith Hosp., London, W12 0HS, UK

SO Biochemical Society Transactions (1988), 16(2), 143-4

CODEN: BCSTB5; ISSN: 0300-5127

DT Journal; General Review

LA English

AB A review with 20 refs. of tech. advances in immunoblotting with regard to the detection of ***Mycobacterium*** *leprae* glycolipid and protein antigens of diagnostic and immunopathogenic interest.

L6 ANSWER 111 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1988:20242 CAPLUS

DN 108:20242

TI Most ***Mycobacterium*** *leprae* carbohydrate-reactive monoclonal antibodies are directed to lipoarabinomannan

AU Gaylord, Harvey; Brennan, Patrick J.; ***Young, Douglas B.*** ;
 Buchanan, Thomas M.
 CS Dep. Microbiol., Colorado State Univ., Fort Collins, CO, 80523, USA
 SO Infection and Immunity (1987), 55(11), 2860-3
 CODEN: INFIBR; ISSN: 0019-9567
 DT Journal
 LA English
 AB Each of >30 monoclonal antibodies that had been raised against *M. leprae* and previously classified as reactive with carbohydrate was shown to be directed against lipoarabinomannan, a prominent, highly pervasive, myo-inositol-phosphate-contg., cross-reactive antigen within the leprosy bacillus. Some of the antibodies preferentially bound to the lipopolysaccharide of *M. leprae* rather than to that of *M. tuberculosis*, suggesting the presence of distinguishing structural features. The presence of alkali-labile inositol 1-phosphate in the lipopolysaccharide from *M. tuberculosis* and its apparent absence from the *M. leprae* product may account for the difference.

L6 ANSWER 112 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1987:418862 CAPLUS
 DN 107:18862
 TI Screening of a recombinant ***mycobacterial*** DNA library with polyclonal antiserum and molecular weight analysis of expressed antigens
 AU ***Young, Douglas B.*** ; Kent, Lenore; Young, Richard A.
 CS MRC Tuberc. Related Infect. Unit, Hammersmith Hosp., London, W12 0HS, UK
 SO Infection and Immunity (1987), 55(6), 1421-5
 CODEN: INFIBR; ISSN: 0019-9567
 DT Journal
 LA English
 AB A .lambda.gt11 library contg. recombinant DNA from ***Mycobacterium*** tuberculosis was screened using hyperimmune anti-*M. tuberculosis* rabbit serum. The majority (22 of 29) of the recombinant clones selected by using polyclonal serum expressed 3 antigens that were previously identified by using mouse monoclonal antibodies, thus indicating the immunodominance of these proteins. Western blot anal. of the recombinant clones demonstrated that expression of these antigens is frequently independent of the formation of .beta.-galactosidase fusion proteins. The mol. wt. of each expressed antigen can vary between clones and is not presently identical to that found in ***mycobacterial*** exts.

L6 ANSWER 113 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1987:421642 CAPLUS
 DN 107:21642
 TI Mapping of T cell epitopes using recombinant antigens and synthetic peptides
 AU Lamp, Jonathan R.; Ivanyi, Juraj; Rees, Ann D. M.; Rothbard, Jonathan B.; Howland, Kevin; Young, Richard A.; ***Young, Douglas B.***
 CS MRC Tuberculosis Related Infect. Unit, Hammersmith Hosp., London, W12 0HS, UK
 SO EMBO Journal (1987), 6(5), 1245-9
 CODEN: EMJODG; ISSN: 0261-4189
 DT Journal
 LA English
 AB Two complementary approaches were used to det. the epitope specificity of clonal and polyclonal human T lymphocytes reactive with the 65-kilodalton (kd) antigen of ***Mycobacterium*** leprae. A recombinant DNA sublibrary constructed from portions of the 65-kd gene was used to map T

cell determinants within amino acid sequences 101-146 and 409-526. Independently, potential T cell epitopes within the protein were predicted based on an empirical anal. of specific patterns in the amino acid sequence. Of 6 peptides that were predicted and subsequently synthesized, 2 (112-132 and 437-459) were shown to contain human T cell epitopes. This corroborated and refined the results obtained using the recombinant DNA sublibrary. Both of these regions are identical in *M. leprae* and *M. tuberculosis* and are distinct from the known B cell epitopes of the 65-kd protein. This combination of recombinant DNA technol. and peptide chem. may prove valuable in anal. of the cellular immune response to infectious agents.

L6 ANSWER 114 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1987:552290 CAPLUS
 DN 107:152290
 TI The 65kDa antigen of ***mycobacteria*** - a common bacterial protein?
 AU ***Young, Douglas B.*** ; Ivanyi, Juraj; Cox, Josephine H.; Lamb, Jonathan R.
 CS R. Postgrad. Med. Sch., Hammersmith Hosp., London, W12 0HS, UK
 SO Immunology Today (1987), 8(7-8), 215-19
 CODEN: IMTOD8; ISSN: 0167-4919
 DT Journal; General Review
 LA English
 AB A review with 23 refs. The 65 kilodalton (kDa) antigen of ***Mycobacterium*** tuberculosis and *M. leprae* is a well-characterized, strongly immunogenic protein eliciting antibody and T-cell responses in infected patients. Recent studies have disclosed regions of cross-reactivity between the 65kDa antigen and proteins in many other bacterial species. These include the product of the *ams* gene in *Escherichia coli* which is involved in the processing of RNA. The significance of the 65kDa antigen and its possible role in the pathogenesis of ***mycobacterial*** and other diseases is discussed.

L6 ANSWER 115 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1987:212114 CAPLUS
 DN 106:212114
 TI T cell activation by anti-idiotypic antibody: mechanism of interaction with antigen-reactive T cells
 AU Rees, Ann D. M.; Scoging, Anne; Dobson, Nicola; Praputpittaya, Kraingsak; ***Young, Douglas*** ; Ivanyi, Juraj; Lamb, Jonathan R.
 CS MRC Tuberc. Related Infect. Unit, Hammersmith Hosp., London, W12 0HS, UK
 SO European Journal of Immunology (1987), 17(2), 197-201
 CODEN: EJIMAF; ISSN: 0014-2980
 DT Journal
 LA English
 AB The activation of T cells by an anti-idiotypic antibody (anti-Id) TB71 contg. an internal image of the corresponding ***mycobacterial*** antigen [38,000 kilodaltons (38 kDa)] was achieved by the interaction of anti-Id TB71 with the T-cell receptor complex (CD3/Ti). The accessory cell requirement in this response could not be replaced by anti-Id TB71 coupled to Sepharose beads and was not inhibited by Fc receptor blockade. When taken together with the finding that anti-Id TB71-induced proliferation of a T cell clone was restricted by determinants encoded by the major histocompatibility complex, these findings suggested that anti-Id TB71 was presented to 38-kDa antigen-reactive T cells by the same mechanisms as conventional antigenic determinants. I.e., both stimulated T cells through the CD3/Ti complex and had to be presented in the context

of class II mols. in accessory cells. The finding that the disruption of the integrity of the anti-Id TB71 combining site did not affect T cell responsiveness, although antibody binding was ablated, implied that anti-Id TB71 may be partially degraded and re-expressed with major histocompatibility complex class II determinants.

L6 ANSWER 116 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1985:503073 CAPLUS
DN 103:103073
TI Immunochemical characterization of a protein associated with
Mycobacterium leprae cell wall
AU Gillis, Thomas P.; Miller, Richard A.; ***Young, Douglas B.*** ;
Khanolkar, Saroj R.; Buchanan, Thomas M.
CS Lab. Res. Branch, Natl. Hansen's Dis. Cent., Carville, LA, 70721, USA
SO Infection and Immunity (1985), 49(2), 371-7
CODEN: INFIBR; ISSN: 0019-9567
DT Journal
LA English
AB A panel of 9 monoclonal antibodies to M. leprae were used to characterize a protein antigen of the bacillus. Two monoclonal antibodies (IVD8 and IIIE9) were specific for M. leprae and reacted with an epitope (CWPa) present on a protein mol. assocd. with the cell wall fraction of M. leprae. This protein, designated cell wall-assocd. protein (CWP), lost its immunoreactivity upon treatment with trypsin and had an apparent mol. wt. of 65,000, though addnl. lower-mol.-wt. forms of the protein were obsd. by immunoblotting. Four other cross-reactive epitopes (CWPb, CWPc, CWPd, and CWPe) were defined on the same mol. using 7 independent monoclonal antibodies. Therefore, M. leprae possesses a trypsin-sensitive, heat-stable protein assocd. with the cell wall which contains at least 1 species-specific and 4 cross-reactive antigenic determinants.

L6 ANSWER 117 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1986:103200 CAPLUS
DN 104:103200
TI Cloning and expression of ***mycobacterial*** genes in E. coli
AU ***Young, Douglas B.***
CS MRC Tuberculosis Related Infect. Unit, Hammersmith Hosp., London, W12 0HS, UK
SO Immunology Today (1985), 6(10), 296-7
CODEN: IMTOD8; ISSN: 0167-4919
DT Journal; General Review
LA English
AB A review with 17 refs. on cloning of genes of ***Mycobacterium*** leprae and M. tuberculosis with phage .lambda. and expression of the cloned genes in Escherichia coli.

L6 ANSWER 118 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1985:452347 CAPLUS
DN 103:52347
TI Use of a polysulfone membrane support for immunochemical analysis of a glycolipid from ***Mycobacterium*** leprae
AU ***Young, Douglas B.*** ; Fohn, Melinda J.; Buchanan, Thomas M.
CS Pac. Med. Cent., Univ. Washington, Seattle, WA, 98144, USA
SO Journal of Immunological Methods (1985), 79(2), 205-11
CODEN: JIMMBG; ISSN: 0022-1759
DT Journal

LA English
 AB Polysulfone membranes were used as a solid support for chromatog. and immunoblotting of phenolic glycolipid I from *M. leprae*. These membranes have an advantage over other supports such as nitrocellulose and silica gel in that very little non-specific background binding of antibodies occurs and assays can readily be carried out with IgM antibodies from human sera. An example of use of the polysulfone chromatog. system for detection of phenolic glycolipid I in sera from leprosy patients is described.

L6 ANSWER 119 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1984:66368 CAPLUS
 DN 100:66368
 TI Generation and characterization of monoclonal antibodies to the phenolic glycolipid of ****Mycobacterium**** *leprae*
 AU ***Young, Douglas B.*** ; Khanolkar, Saroj R.; Barg, Linda L.; Buchanan, Thomas M.
 CS Seattle Public Health Hosp., Univ. Washington, Seattle, WA, 98144, USA
 SO Infection and Immunity (1984), 43(1), 183-8
 CODEN: INFIBR; ISSN: 0019-9567
 DT Journal
 LA English
 AB Nine cloned cell lines producing antibodies to the unique phenolic glycolipid of *M. leprae* were established as a result of fusions with spleens from mice immunized with the glycolipid complexed with methylated bovine serum albumin. One of the antibodies was relatively nonspecific, binding to a related glycolipid from *M. kansasii*, but the remaining antibodies were specific for the *M. leprae* lipid. Some of the antibodies required the intact (trisaccharide) carbohydrate portion for recognition of the glycolipid antigen, whereas others recognized partially hydrolyzed forms lacking 1 or 2 sugar residues. Monoclonal antibodies directed at the terminal saccharide of the glycolipid showed the greatest specificity for *M. leprae* in enzyme immunoassays. These antibodies brightly labeled whole ****mycobacteria**** in indirect immunofluorescence expts., demonstrating the surface location of *M. leprae*-specific determinants of the glycolipid antigen. In addn. to their use in providing information about the antigenic properties of the phenolic glycolipid, these antibodies have potential applications for elucidating the roles of glycolipid in the pathogenesis of leprosy.

L6 ANSWER 120 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1981:585029 CAPLUS
 DN 95:185029
 TI Detection of ****mycobacterial**** lipids in skin biopsies from leprosy patients
 AU ***Young, Douglas B.***
 CS Found. Med. Res., Bombay, 400 018, India
 SO International Journal of Leprosy and Other Mycobacterial Diseases (1981), 49(2), 198-204
 CODEN: IJLEAG; ISSN: 0020-7349
 DT Journal
 LA English
 AB TLC was used to compare lipid exts. from lepromatous skin biopsies with those from normal skin and from ****Mycobacterium**** *leprae* purified from armadillo spleen. The *M. leprae* from armadillo spleen showed the same lipid characteristics as bacilli from human skin samples. Several lipids were found in infected skin which were absent from normal skin but

corresponded to lipids present in the purified *M. leprae*. These included mycolic acids, a 6-deoxyhexose-contg. lipid (glycolipid I), and a wax ester (possibly related to the *M. tuberculosis* wax, phthiocerol dimycocerosate). Unlike *M. lepraemurium*, *M. leprae* contained no C-type mycosides. Detn. of ***mycobacterial*** lipids in lepromatous skin biopsies indicated that their concns. were much higher than would be predicted from the no. of acid-fast bacilli present. Perhaps, accumulation of lipid debris from dead *M. leprae* could provide a protective environment in infected cells for remaining viable bacilli.

L6 ANSWER 121 OF 121 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1981:153093 CAPLUS
DN 94:153093
TI Identification of ***Mycobacterium*** leprae: use of wall-bound mycolic acids
AU ***Young, Douglas B.***
CS Found. Med. Res., Bombay, 400018, India
SO Journal of General Microbiology (1980), 121(1), 249-53
CODEN: JGMIAN; ISSN: 0022-1287
DT Journal
LA English
AB Sepn. of wall-bound mycolic acids and anal. according to their functional groups by thin layer chromatog. showed a difference between *M. leprae* and a no. of strains of acid-fast bacilli cultured from leprosy biopsy in vitro. This technique may be of value as a convenient preliminary test for the identification of possible *M. leprae* cultures.

=> s mycobacter? and (heat shock)
L7 6959 MYCOBACTER? AND (HEAT SHOCK)

=> s l7 and (hsp?)
L8 4000 L7 AND (HSP?)

=> dup rem l8
PROCESSING IS APPROXIMATELY 30% COMPLETE FOR L8
PROCESSING IS APPROXIMATELY 67% COMPLETE FOR L8
PROCESSING COMPLETED FOR L8
L9 1842 DUP REM L8 (2158 DUPLICATES REMOVED)

=> s l7 and (grp? or clp? or (alpha cystallin))
L10 267 L7 AND (GRP? OR CLP? OR (ALPHA CYSTALLIN))

=> dup rem l10
PROCESSING COMPLETED FOR L10
L11 239 DUP REM L10 (28 DUPLICATES REMOVED)

=> s l9 and (modified(3w)product?)
5 FILES SEARCHED...
L12 14 L9 AND (MODIFIED(3W) PRODUCT?)

=> s l11 and (modified(3w)product?)
L13 9 L11 AND (MODIFIED(3W) PRODUCT?)

=> d l12 bib ab kwic 1-
YOU HAVE REQUESTED DATA FROM 14 ANSWERS - CONTINUE? Y/(N):y

L12 ANSWER 1 OF 14 USPATFULL on STN

AN 2003:237907 USPATFULL

TI Compositions and methods for the therapy and diagnosis of colon cancer

IN King, Gordon E., Shoreline, WA, UNITED STATES

Meagher, Madeleine Joy, Seattle, WA, UNITED STATES

Xu, Jiangchun, Bellevue, WA, UNITED STATES

Secrist, Heather, Seattle, WA, UNITED STATES

Jiang, Yuqiu, Kent, WA, UNITED STATES

PA Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)

PI US 2003166064 A1 20030904

AI US 2002-99926 A1 20020314 (10)

RLI Continuation-in-part of Ser. No. US 2001-33528, filed on 26 Dec 2001,
PENDING Continuation-in-part of Ser. No. US 2001-920300, filed on 31 Jul
2001, PENDING

PRAI US 2001-302051P 20010629 (60)

US 2001-279763P 20010328 (60)

US 2000-223283P 20000803 (60)

DT Utility

FS APPLICATION

LREP SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300,
SEATTLE, WA, 98104-7092

CLMN Number of Claims: 17

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 8531

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compositions and methods for the therapy and diagnosis of cancer,
particularly colon cancer, are disclosed. Illustrative compositions
comprise one or more colon tumor polypeptides, immunogenic portions
thereof, polynucleotides that encode such polypeptides, antigen
presenting cell that expresses such polypeptides, and T cells that are
specific for cells expressing such polypeptides. The disclosed
compositions are useful, for example, in the diagnosis, prevention
and/or treatment of diseases, particularly colon cancer.

SUMM [2042] For example, certain amino acids may be substituted for other
amino acids ***in*** a protein structure without ***appreciable***
loss of interactive binding capacity with structures such as, for
example, antigen-binding regions of antibodies or binding sites on
substrate molecules. Since it is the interactive capacity and nature of
a protein that defines that protein's ***biological*** functional
activity, ***certain*** ***amino*** acid ***sequence***
substitutions can be made ***in*** a ***protein***
sequence, ***and***, ***of*** course, its underlying

DNA

coding sequence, and nevertheless obtain a protein with like
properties. It is thus contemplated that various changes may be made
in ***the*** ***peptide*** sequences of the disclosed
compositions, or corresponding DNA sequences which encode said peptides
without appreciable loss of their biological utility. . . Cys C
UGC UGU

Aspartic acid Asp D GAC GAU

Glutamic acid Glu E GAA GAG

Phenylalanine Phe F UUC ***UUU***

Glycine Gly G ***GGA*** GGC GGG GGU

Histidine His H CAC CAU

Isoleucine Ile I AUA AUC AUU

Lysine Lys K AAA AAG

Leucine. . .

DETD . . . cDNA:FLJ22083 fis, clone

HEP14459, highly similar to HUM3H3M Homo sapiens 3-hydroxy-3-methylglutaryl coenzymeA synthase

SEQ ID NO: 1801 74815 Homo sapiens , ***heat*** ***shock***
40 kD protein 1, clone

SEQ ID NO: 1802 74816 MGC:8425, mRNA, complete cds
Homo sapiens hypothetical protein FLJ22195 (FLJ22195), mRNA

SEQ. . . 1806 74827 Homo sapiens , ribophorin II, clone MGC:1817, mRNA, complete cds

SEQ ID NO: 1807 74828 Homo sapiens similar to ***HSPC039***
protein

SEQ ID NO: 1808 74829 (H.sapiens) (LOC65818), mRNA
Homo sapiens cell cycle protein CDC20 mRNA, complete cds

SEQ ID NO: 1809. . . Alg5, S. cerevisiae, homolog of (ALG5), mRNA

SEQ ID NO: 1819 74854 Human cis-acting sequence

SEQ ID NO: 1820 74856 Homo sapiens ***HSPC128*** protein (
HSPC128),

SEQ ID NO: 1821 74857 mRNA
Homo sapiens cDNA FLJ11051 fis, clone PLACE1004629, weakly similar to PROTEIN OS-9 PRECURSOR

SEQ ID NO:. . .

L12 ANSWER 2 OF 14 USPATFULL on STN

AN 2003:225302 USPATFULL

TI Compositions and methods for treatment of neoplastic disease

IN Terman, David S., Pebble Beach, CA, UNITED STATES

PI US 2003157113 A1 20030821

AI US 2000-751708 A1 20001228 (9)

PRAI US 1999-173371P 19991228 (60)

DT Utility

FS APPLICATION

LREP David S. Terman, P.O. Box 987, Pebble beach, CA, 93953

CLMN Number of Claims: 60

ECL Exemplary Claim: 1

DRWN 3 Drawing Page(s)

LN.CNT 15804

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention comprises compositions and methods for treating a tumor or neoplastic disease in a host, The methods employ conjugates comprising superantigen polypeptides, nucleic acids with other structures that preferentially bind to tumor cells and are capable of inducing apoptosis. Also provided are superantigen-glycolipid conjugates and vesicles that are loaded onto antigen presenting cells to activate

both T cells and NKT cells. Cell-based vaccines comprise tumor cells engineered to express a superantigen along with glycolipids products which, when expressed, render the cells capable of eliciting an effective anti-tumor immune response in a mammal into which these cells are introduced. Included among these compositions are tumor cells, hybrid cells of tumor cells and accessory cells, preferably dendritic cells. Also provided are tumoricidal T cells and NKT cells devoid of inhibitory receptors or inhibitory signaling motifs which are hyperresponsive to the the above compositions and lipid-based tumor associated antigens that can be administered for adoptive immunotherapy of cancer and infectious diseases.

SUMM . . . cell both enterotoxins and --.alpha.-galactosylceramides are associated with numerous intracellular and membrane structures such as MHC, costimulatory and adhesion molecules, ***heat*** ***shock*** proteins, membrane glycolipids and glycosphingolipids which may improve immunogenicity and antigen presentation. They may also be transported in various vesicles. . . .

SUMM . . . structures may actually improve the T cell activating function of SAGs such as deoxyribonucleic acids, ribonucleic acids, tumor associated antigens, ***heat*** ***shock*** proteins, costimulatory molecules and adhesion molecules and endosomes. Cellular SAG peptides or nucleotides exist in association with tumor associated antigens, costimulants, adhesion molecules, ***heat*** ***shock*** proteins and MHC molecules, GPI-ceramides or SAG receptors (digalactosylceramides) which improve the immunogenicity of the tumor antigens. Therefore, these structural. . . .

SUMM . . . tumor cells, an immunogenic bacterial product such as Staphylococcal adhesin protein A, LPS, .beta.-glucans, and peptidoglycans, costimulatory and adhesion molecules, ***heat*** ***shock*** protein, growth factor receptors such as Her/neu and

tumor markers such as PSA.

DETD [0074] 19. ***Heat*** - ***shock*** proteins, ATPases and G

DETD . . . the ability to stimulate large subsets of T cells. SAGs include Staphylococcal enterotoxins, Streptococcal pyrogenic exotoxins, Mycoplasma antigens, rabies antigens, ***mycobacteria*** antigens, EB viral antigens, minor lymphocyte stimulating antigen, mammary tumor virus antigen, ***heat*** ***shock*** proteins, stress peptides, clostridial and toxoplasmosis antigens and the like. Any SAG can be used as described herein, although, Staphylococcal. . . .

DETD . . . in gram positive bacteria (such as Staphylococcus or Streptococcus), to nucleic acids encoding capsular polysaccharides and teichoic acids and in ***mycobacterial*** species to nucleic acids encoding lipoarabinan.

DETD . . . E. coli, Salmonella or Klebsiella or for bacteria which naturally produce lipoarabinomannans glycans or polysaccharides containing cell walls such as ***Mycobacterium*** and Streptococcus respectively. The SAG-polysaccharide constructs bind to CD1 receptors of antigen presenting cells. They are then capable of activating. . . .

DETD . . . J et al., J. Natl. Cancer Inst. 72:955-962 (1984)). By synthesizing single stranded nucleotides corresponding to different regions in the ***Mycobacterium*** bovis genome, specific single stranded oligonucleotides that activate adherent splenocytes and enhanced natural killer cell activity have been identified. In. . . .

DETD . . . response. Examples are LPS's of gram negative organisms, SAGs and peptidoglycans of gram positive organisms, fungal .beta.-glucans,

bacterial glycosylceramides, and ***mycobacterial*** lipoarabinans. Numerous infectious agents with these structures cause potent immune reactions e.g. streptococcal cellulitis induced by *S. pyogenes*, *E. coli*.

- DETD . . . molecule interact with different types of host cells. There is also evidence that immunopotentiating activity of a glycopeptide produced by ***mycobacteria*** is dependent on the saccharide residues of the molecule. The capsular polysaccharide of the *Streptococcus* is extremely immunogenic, consisting of. . .
- DETD [0349] Genes Involved in ***Mycobacterial*** Cell Wall Biosynthesis
- DETD . . . fused in frame or cotransfected into tumor cell with nucleic acids encoding the key enzymes involved in the biosynthesis of ***mycobacterial*** cell wall mycolic acid, phosphatidylinositol mannosides and lipoarabinans. A high affinity interaction of CD1b molecules with the acyl side chains. . .
- DETD . . . in the biosynthesis of these molecules have been isolated. In addition to the usual fatty acids found in membrane lipids, ***mycobacteria*** have a wide variety of very long-chain saturated (C18-C32) and monounsaturated (up to C26) n-fatty acids. The occurrence of a-alkyl b-hydroxy very long chain fatty acids i e., mycolic acid is a hallmark of ***mycobacteria*** and related species. ***Mycobacterial*** mycolic acids are the largest (C70-C90) with the largest-branch (C20-C25). The main chain contains one or two double bonds, cyclopropane. . . esterified to glycerol or sugars such as trehalose, glucose and fructose depending on the sugars present in the culture medium. ***Mycobacterium*** also contains several methyl-branched fatty acids. These include 10-methyl C18 fatty acid (tuberculostearic acid found esterified in phosphatidyl inositide mannosides),. . .
- DETD [0355] The MAS gene encoding ***mycobacterial*** mycocerosic acid synthase is a dimer of the FAS gene. The cloning and sequencing of the MAS gene revealed the. . .
- DETD . . . results in tissue specific forms of the protein, which can be intracellular, membrane bound, or secreted. In cells infected with ***mycobacteria***, the CD1 molecule binds and presents a ***mycobacterial*** membrane component, mycolic acid. Surface CD1 molecules present longer peptides than those normally found on class I molecules. Whether CD1. . .
- DETD . . . Nucleic acid encoding cell wall or cell membrane associated glycosylceramides or a branched, b hydroxy long-chain fatty acids found in ***mycobacteria*** and other bacteria are cotransfected into the CD1 transfected tumor cells. The tumor cell therefore displays glycosylceramides bound to the. . .
- DETD [0460] 36. SAGs Combined with Signal Transduction Molecules or ***Heat*** ***Shock*** Proteins (***HSPs***)
- DETD . . . frame to (or cotransfected with) a nucleic acid encoding "signal transduction molecules" such as Ras, JAK 1 and STAT-1a and ***heat*** ***shock*** proteins ***HSP*** -60, ***HSP*** -70, ***HSP*** -90a, ***HSP*** -90b, Cox-2 as well as heterotrimeric G proteins and ATPases. The genes for *Staphylococcal* ***HSP*** -70 useful in this invention have been cloned (Ohta, T et al., *J. Bacteriology* 176: 4779-4783, (1994)). As used herein, SAG. . . of above structures at the peptide or nucleic acid level. Preferred proteins for this embodiment are G proteins, ATPases and ***HSPs***. Chemical conjugation is carried out by conventional methods, e.g., use of preferred heterobifunctional crosslinkers. Alternatively, conjugates are produced genetically as. . .

DETD [0462] SAg-encoding nucleic acid is fused in frame (or cotransfected) with nucleic acid encoding a signal transduction protein or. ***HSP***. Transfectants are prepared as in Example 1. They are used in vivo as a preventative or therapeutic antitumor vaccine according.

DETD . . . 37. SAGs with Specialized Sites for C-terminal GPI Anchoring, Glycosylation, Sulfation, N-Myristoylation, Phosphorylation, Hydroxylation N-Methylation, Signal Peptide Binding, LPS Binding, ***HSP*** Binding, Chemokine Binding and Prenylation

DETD [0469] Nucleic acids encoding ***HSPs***, along with their promoters, are fused in-frame (or cotransfected) with SAG nucleic acid. These include but are not limited to two recently discovered ***HSP*** genes, orf37 and orf 35 in *Staphylococcus aureus* that are upstream and downstream of *grpE*(***hsp20***), *dnaK*(***hsp70***) and *dnaJ*(***hsp40***) in the following sequence: orf37-- ***hsp20*** -- ***hsp70*** -- ***hsp40*** --orf35. The promoters are located upstream of orf37 and upstream of ***hsp40***. These fused proteins are useful as preventative or therapeutic antitumor vaccines according to Examples 15, 16, 18-23. They are also.

DETD . . . (in terms of number) SAG receptor site binds to tumor cells expressing SAG receptors. SAGs possess a site for binding ***HSPs*** which are useful in immunizing normal or anergic T cells in a tumor patient. SAGs bind to T cell antagonist.

DETD . . . Furthermore, nucleic acids encoding proteins listed in Tables I, II, IV and V, for example, angiostatin, protein A, erb/Neu and ***HSPs***, staphylococcal collagen adhesin, are introduced into and expressed in tumor cells or DCs that express or secrete SAG, or into.

DETD . . . pathway. Owing to the lower B-carotene content, Lp(a) may be more easily oxidized than LDL. Oxidized Lp(a) such as Lp(a) ***modified*** by malondialdehyde, a ***product*** generated in vivo from aggregated platelets, is avidly taken up by monocyte-macrophages. through the scavenger-receptor pathway. Lp(a) accumulates in either.

DETD . . . specifically to LBTAAs which include fatty acids, ceramides, glycolipids, sphingolipids, glycosphingolipids, phtosphingolipids, gangliosides, lipopeptides. IRIDAS recognize LBIDAS derived from bacteria, ***mycobacteria***, parasites, fungi, protozoans or plants and respond by producing an effective immunocyte response. These antigens comprise sphingolipids, glycopeptides, phytoglycolipids, mycoglycolipids, . . .

DETD . . . acids, ceramides, glycolipids, sphingolipids, glycosphingolipids, gangliosides, lipopeptides. Superantigens are also conjugated to LBIDAS, glycan and peptidoglycan antigens derived from bacteria, ***mycobacteria***, parasite, fungi or plants comprising sphingolipids, glycopeptides, peptidoglycans and teichoic acids, phytoglycolipids, mycoglycolipids, lipoarabinan, mycolic acids, Braun's lipopeptide, inositolphosphorylceramides and. . . given in Examples 15, 16, 21, 23, 53, 54. Conjugates consisting of SAG and LBIDAS derived from fungal, parasitic or ***mycobacterial*** sources are also useful for the treatment of infectious diseases such as tuberculosis, leishmaniasis, trypanosomiasis as given in Example 53. . . a population of immunocytes with deleted (via gene knockout) or functionally inactivated (antisense) IRIDAS specific for bacterial, fungal, parasitic or ***mycobacterial*** antigens for use in adoptive immunotherapy of infectious disease (Examples 51, 52, 53).

31. Lipid A biosynthetic (SEQ ID NOS: 105-112) Tumor
 Schnaitman CA et al.,
 genes lpxA-D
 Microbiological Reviews 57:
 655-682 (1993)

32. ***Mycobacterial*** mycolic acid (SEQ ID NOS: 113-114) Tumor
 Fernandes ND et al., Gene
 biosynthetic genes
 170: 95-99 (1996); Mathur M
 et al., J.Biol. . . .

DETD . . . and greatly restricted anchors are preferred. This recognition
 of CD1-presented antigens depends on the type and distribution of sugar
 residues. ***Mycobacterial*** cell wall antigens namely mycolic
 acids and lipoarabinomannan also bind to CD1. Recently several
 glycosylceramides, in particular, monogalactosyl ceramides GalCer). .

DETD . . . or exosomes comprising SAg-GalCer complexes or SAg-tumor
 peptide (including but not limited to normal mutated structures). The
 ternary complexes of SAg-GalCer- ***heat*** ***shock*** protein
 and tumor peptide- ***heat*** ***shock*** protein are also
 useful, These complexes may be in or soluble or immobilized form,
 attached to a CD1 or MHC. . . .

DETD . . . acid and growth factor receptor nucleic acids

18. SAg-encoding nucleic acid and cell cycle protein nucleic acids

19. SAg-encoding nucleic acid and ***heat*** ***shock*** protein
 nucleic acids

20. SAg-encoding nucleic acid and chemokine nucleic acids

21. SAg-encoding nucleic acid and cytokine nucleic acids

22. SAg-encoding nucleic acid. . . .

DETD . . . molecules containing amino acid sequences and homologous to the
 enterotoxin family of molecules. To this extent, mammary tumor virus
 sequences, ***heat*** ***shock*** proteins, stress peptides,
 Mycoplasma and ***mycobacterial*** antigens, and minor lymphocyte
 stimulating loci bearing tumoricidal structural homology to the
 enterotoxin family are useful as anti-tumor agents. Hybrid. . . .

DETD . . . commonly used inducible promoters include the metallothionein
 CUP1 promoter, which is tightly controlled by copper; promoters
 activated in response to ***heat*** ***shock*** , which are of
 particular interest for expression in the temperature-sensitive sec6-4
 mutant and the PH05 promoter, which is derepressed at. . . .

DETD [1886] C57 BL/6 mice are used. These mice are natural-killer-cell-
 deficient. Beige mice are infected with many of the nontuberculous
 mycobacteria : MAC, M. kansasii, M. simiac, M. malmoense and M.
 genavense. Same-sex mice 5-7 weeks old are allowed to acclimate for. .

DETD [1888] Primary cultures of MAC (M. kansasii or other
 mycobacteria) to be used for infection are obtained from
 clinical isolates of patients with disseminated MAC infection, or the
 American Type. . . .

DETD [1955] Preparation of Lipid-Based Tumor Associated Antigens (LBTAAs) &
 Lipid-Based Infectious Disease Associated Antigens (LBIDAS) of
 Bacterial, Fungal, Yeast, Parasitic, ***Mycobacterial*** ,
 Invertebrate and Protozoan Origin

CLM What is claimed is:
 2 The receptor of claim 1 wherein the lipid antigen is a bacterial,
 fungal, protozoal or ***mycobacterial*** antigen.

. . . cell wherein said receptor inhibits cellular activation by receptors specific for lipid-based infectious disease associated antigens derived from bacteria, fungi, ***mycobacterium***, parasite, virus, eukaryote or prokaryote antigens in the context of MHC or CD1.

11. The lipid antigens derived from bacteria, ***mycobacteria***, fungi and protozoa marine invertebrates of claim 2 wherein said lipid antigens are selected from the group consisting of glycosylceramides, .

. . . based inhibitory motifs which inhibits cellular activation by receptors specific for lipid-based infectious disease associated antigens derived from bacteria, fungi, ***mycobacteria***, parasite, virus, eukaryote or prokaryote antigens are deleted or functionally deactivated.

. . . of claims 24-29 wherein said superantigen comprises a staphylococcal enterotoxin, a streptococcal pyrogenic exotoxin, mycoplasma arthritides, rabies virus, clostridial antigen, ***heat*** ***shock*** protein.

L12 ANSWER 3 OF 14 USPATFULL on STN

AN 2003:152692 USPATFULL

TI Diagnosis methods based on microcompetition for a limiting GABP complex

IN Polansky, Hanan, Rochester, NY, UNITED STATES

PI US 2003104358 A1 20030605

AI US 2002-219649 A1 20020815 (10)

RLI Continuation-in-part of Ser. No. US 2000-732360, filed on 7 Dec 2000, PENDING

DT Utility

FS APPLICATION

LREP Hanan Polansky, 3159 S. Winton Rd., Rochester, NY, 14623

CLMN Number of Claims: 32

ECL Exemplary Claim: 1

DRWN 28 Drawing Page(s)

LN.CNT 14430

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Microcompetition for GABP between a foreign polynucleotide and cellular GABP regulated genes is a risk factor associated with many chronic diseases such as obesity, cancer, atherosclerosis, stroke, osteoarthritis, diabetes, asthma, and other autoimmune diseases. The invention uses this novel discovery to present assays for the diagnosis of these chronic diseases. The assays are based on measuring the cellular copy number of the foreign polynucleotide, measuring the rate of complex formation between GABP and either the foreign polynucleotide, or a cellular GABP regulated gene, identifying modified expression of a cellular GABP regulated gene, or identifying modified activity of the gene product of a GABP regulated gene. The invention also presents other foreign polynucleotide-type assays.

DETD . . . ExoKII, FAS, TSP-1, FGF-4, .alpha.1-chim, Tr Hydr, NaKATPsea-3, PDGF.beta., FerH, MHC IA2 B8, Cw2Ld and B7, MDR1, CYPLA1, c-JUN, Grp78, ***Hsp70***, ADH2, GPAT, FPP, HMG, HSS, SREBP2, GHR, CP2, .beta.-actin, TK, TopoII.alpha., I, II, III, IV, cdc25, cdc2, cyclA, cyclB1, E2F1, . . .

DETD . . . DNA sample, one using primers specific for the DNA prior to bisulfite treatment, and one using primers for the chemically ***modified*** DNA. The amplification ***products*** are resolved

on native polyacrylamide gels and visualized by staining with ethidium bromide followed by UV illumination. Amplification products detected.

- DETD . . . islets compared with various other mouse tissues (Lenzen 1996.sup.548). Moreover, induction of cellular stress by high glucose, high oxygen, and ***heat*** ***shock*** treatment did not affect antioxidant enzyme expression in rat pancreatic islets or in RINm5F insulin-producing cells (Tiedge 1997.sup.549). Based on. . .
- DETD . . . copy number of viral genome in the subsequent latent period. Infection with some viruses, such as measles, hepatitis A, and ***Mycobacterium*** tuberculosis induce a strong polarized Th1-type response in early life. These infections reduce GABP virus replication and subsequent genome copy. . .
- DETD [2078] BCG is a freeze-dried preparation made from a living culture of the Calmette-Guerin strain of ***mycobacterium*** Bovis. It was first developed as a vaccine against tuberculosis in 1921 but also has been used as an immunotherapeutic. . .
- DETD [2079] Results of numerous studies suggest that measles, hepatitis A, and ***Mycobacterium*** tuberculosis infection in early life may prevent subsequent development of atopic diseases. In humans, immunomodulation during the first two years. . .
- DETD [2081] Another study showed that an infection of NOD mice with ***Mycobacterium*** avium, before the mice show overt diabetes, results in permanent protection of the animals from diabetes. This protective effect was. . .
- DETD . . . V V, Nakatani Y, Wolffe A P. Xenopus NF-Y pre-sets chromatin to potentiate p300 and acetylation-responsive transcription from the Xenopus ***hsp70*** promoter in vivo. EMBO J. 1998 Nov 2; 17(21): 6300-15.
- .sup.39 Faniello M C, Bevilacqua M A, Condorelli G, de Crombrughe B,. . .
- DETD . . . T, Bennett S, Wheeler J, Huygen K, Aaby P, McAdam K P, Newport M J. Newborns develop a Th1-type immune response to ***Mycobacterium*** bovis bacillus Calmette-Guerin vaccination. J Immunol. 1999 Aug 15; 163(4): 2249-55.
- .sup.776 Starr S E, Visintine A M, Tomeh M O,. . . of symptoms of asthma, rhinitis, and eczema. Thorax 2000 Jun; 55(6): 449-53.
- .sup.780 von Hertzen L, Klaukka T, Mattila H, Haahtela T. ***Mycobacterium*** tuberculosis infection and the subsequent development of asthma and allergic conditions. J Allergy Clin Immunol. 1999 Dec; 104(6): 1211-4.
- .sup.781 Scanga C B,. . . 1 diabetes mellitus: is there a link? Drug Saf. 1999 Mar; 20(3): 207-12.
- .sup.786 Martins T C, Aguas A P. Mechanisms of ***Mycobacterium*** avium-induced resistance against insulin-dependent diabetes mellitus (IDDM) in non-obese diabetic (NOD) mice: role of Fas and Th1 cells. Clin Exp Immunol 1999 Feb; 115(2): 248-54.
- .sup.787 Bras A, Aguas A P. Diabetes-prone NOD mice are resistant to ***Mycobacterium*** avium and the infection prevents autoimmune disease. Immunology. 1996 Sep; 89(1): 20-5.
- .sup.788 Pabst H F, Spady D W, Pilarski L M,. . .

TI Method
 IN Kozlowski, Roland, Babraham, UNITED KINGDOM
 McAndrew, Michael B., Babraham, UNITED KINGDOM
 Blackburn, Jonathan Michael, Cambridge, UNITED KINGDOM
 Mulder, Michelle Anne, Cambridge, UNITED KINGDOM
 Samaddar, Mitali, Cambridge, UNITED KINGDOM
 PA Sense Proteomic Limited (non-U.S. corporation)
 PI US 2003073811 A1 20030417
 AI US 2002-114334 A1 20020403 (10)
 RLI Continuation-in-part of Ser. No. WO 2001-GB3693, filed on 17 Aug 2001,
 UNKNOWN
 PRAI GB 2000-20357 20000817
 US 2000-247995P 20001114 (60)
 DT Utility
 FS APPLICATION
 LREP STERNE, KESSLER, GOLDSTEIN & FOX PLLC, 1100 NEW YORK AVENUE, N.W., SUITE
 600, WASHINGTON, DC, 20005-3934
 CLMN Number of Claims: 66
 ECL Exemplary Claim: 1
 DRWN 6 Drawing Page(s)
 LN.CNT 2055

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to novel methods of producing proteins in
 which one or more domains are full length and correctly folded and which
 are each tagged at either the N- or C-terminus with one or more marker
 moieties and arrays containing such proteins, as well as the use of such
 proteins in arrays for rapid screening.

SUMM . . . genome is .about.5 Mbp and a small number have now been
 completely sequenced (for example Helicobacter pylori, Escherichia coli,
 and ***Mycobacterium*** tuberculosis); fungal genomes are typically
 .about.40 Mbp, mammalian genomes at .about.3 Gbp and plant genomes at
 .about.10 Gbp. Current estimates. . .

DETD [0118] (e) Cloning and Analysis of the ***Modified***
 Products (see FIG. 3).

DETD . . . sequencing.

	Accession No
Open Reading Frame	
Homo sapiens ribosomal protein S8 (RPS8)	NM_001012
Homo sapiens golgi autoantigen (with transmembrane signal), 1 (GOLGB1)	NM_004487
Homo sapiens ***heat*** ***shock*** 90 kD protein 1, alpha (***HSPCA*** NM_005348	
Homo sapiens amyloid beta (A4) precursor protein (protease nexin-II, Alzheimer disease) (APP)	NM_000484
H sapiens eukaryotic translation elongation factor 1 delta BC012819 (guanine. . . H. sapiens similar to signal recognition particle XM_057253	
Homo sapiens ribosomal protein, large, P0 (RPLP0), transcript variant 1	NM_001002
Human heart mRNA for ***heat*** ***shock*** protein 90 D87666	
Human DNA sequence from clone RP3-388E23 on chromosome 6q22.33-24.1	
Homo sapiens PDZ domain protein (Drosophila inaD-like) (INADL),	NM_005799
Homo sapiens hypothetical gene. . .	

L12 ANSWER 5 OF 14 USPATFULL on STN
 AN 2003:106233 USPATFULL
 TI Compositions and methods for the therapy and diagnosis of pancreatic cancer
 IN Benson, Darin R., Seattle, WA, UNITED STATES
 Kalos, Michael D., Seattle, WA, UNITED STATES
 Lodes, Michael J., Seattle, WA, UNITED STATES
 Persing, David H., Redmond, WA, UNITED STATES
 Hepler, William T., Seattle, WA, UNITED STATES
 Jiang, Yuqiu, Kent, WA, UNITED STATES
 PA Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)
 PI US 2003073144 A1 20030417
 AI US 2002-60036 A1 20020130 (10)
 PRAI US 2001-333626P 20011127 (60)
 US 2001-305484P 20010712 (60)
 US 2001-265305P 20010130 (60)
 US 2001-267568P 20010209 (60)
 US 2001-313999P 20010820 (60)
 US 2001-291631P 20010516 (60)
 US 2001-287112P 20010428 (60)
 US 2001-278651P 20010321 (60)
 US 2001-265682P 20010131 (60)
 DT Utility
 FS APPLICATION
 LREP SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300, SEATTLE, WA, 98104-7092
 CLMN Number of Claims: 17
 ECL Exemplary Claim: 1
 DRWN No Drawings
 LN.CNT 14253
 CAS INDEXING IS AVAILABLE FOR THIS PATENT.
 AB Compositions and methods for the therapy and diagnosis of cancer, particularly pancreatic cancer, are disclosed. Illustrative compositions comprise one or more pancreatic tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly pancreatic cancer.
 SUMM [2043] SEQ ID NO:2003 is the determined cDNA sequence of clone 61496359

DETD . . . and Ubiqu.-

			binding domains
270	PNCM-86	72179	Hu. Protein A kinase (PRKA) anchor
	protein		
			(gravin) 12 (AKAP12)
271, 272	PNCM-87	73421	Hu. ***heat*** ***shock***
	105kD, antigen NY-CO-25		
273	PNCM-88	72180	Hu. ***heat*** ***shock***
	105kD, antigen NY-CO-25 (Colon		
			cancer Ag.)
274	PNCM-89	72181	Hu. ferritin, heavy polypeptide 1
	(FTH1)		
275	PNCM-90	72182	Hu. frizzled (Drosophila) homolog.
	PNCM-95	72187	Hu. kinectin 1 (kinesin receptor) (KTN1)
	[bp 813-		
			1223]

281	PNCM-96	72188	Hu. prosaposin [bp 608-1018]
282	PNCM-97	72189	Hu. ***heat*** ***shock***
	105kD . . . [bp 1-412]		
283	PNCM-98	72190	Hu. clone IMAGE:3449323
284	PNCM-99	72191	Hu. rabaptin-5 [bp 1578-1990]
285	PNCM-100	72192	Hu. . . . fis, clone LNG01826
298	PNCM-119	72205	Hu. cDNA DKFZp586F1918
299, 300	PNCM-120	72206	Macaca fascicularis brain cDNA, clone
	QflA-11332		
301	PNCM-122	73422	Hu. ***heat*** ***shock***
	105kD, antigen NY-CO-25		
302	PNCM-123	73423	Hu. IMAGE:3355762, chromodomain
	helicase		
			DNA binding protein 1-like
303	PNCM-124	73424	Hu. kinectin 1 (kinesin receptor). .
	. 74602 Hu. fer-1 (C. elegans)-like 3 (myoferlin) (FER1L3)		
337	PNCM-148	73445	Hu. prosaposin (PSAP), sphingolipid
	activator		
			protein 1
338	PNCM-150	73456	Hu. ***heat*** ***shock***
	105kD, antigen NY-CO-25		
339	PNCM-151	73585	Hu. ***heat*** ***shock***
	105kD (***HSP*** -105B)		
340, 341	PNCM-152	73586	Hu. Protein A kinase (PRKA) anchor
	protein		
			(gravin) 12
342, 343	PNCM-153	73587	Hu. cleavage stimulation factor,
	subunit. . .		

L12 ANSWER 6 OF 14 USPATFULL on STN

AN 2003:100088 USPATFULL

TI Treatment methods based on microcompetition for a limiting GABP complex

IN Polansky, Hanan, Rochester, NY, UNITED STATES

PI US 2003069199 A1 20030410

AI US 2002-219334 A1 20020815 (10)

RLI Continuation-in-part of Ser. No. US 2000-732360, filed on 7 Dec 2000,
PENDING

DT Utility

FS APPLICATION

LREP Hanan Polansky, 3159 S. Winton Rd., Rochester, NY, 14623

CLMN Number of Claims: 26

ECL Exemplary Claim: 1

DRWN 28 Drawing Page(s)

LN.CNT 14837

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Microcompetition for GABP between a foreign polynucleotide and a cellular GABP regulated gene is a risk factor associated with chronic disease such as obesity, cancer, atherosclerosis, stroke, osteoarthritis, diabetes, asthma, and other autoimmune diseases. The invention uses this novel discovery to present methods for the treatment of these chronic diseases. The methods are based on modifying such microcompetition, or the effect of such microcompetition on the cell. For instance, treatment may modify the cellular copy number of the foreign polynucleotide, change the rate of complex formation between GABP and either the foreign polynucleotide or the cellular GABP regulated gene, vary the expression of the cellular GABP regulated gene, or manipulate the activity of the gene product of the cellular GABP

regulated gene. The invention also presents methods for treatment of chronic diseases resulting from other foreign polynucleotide-type disruptions.

- DETD . . . ExoKII, FAS, TSP-1, FGF-4, .alpha.1-chim, Tr Hydr, NaKATPsea-3, PDGF.beta., FerH, MHC IA2 B8, Cw2Ld and B7, MDR1, CYP1A1, c-JUN, Grp78, ***Hsp70***, ADH2, GPAT, FPP, HMG, HSS, SREBP2, GHR, CP2, .beta.-actin, TK, TopoII.alpha., I, II, III, IV, cdc25, cdc2, cyclA, cyclB1, E2F. . .
- DETD . . . DNA sample, one using primers specific for the DNA prior to bisulfite treatment, and one using primers for the chemically ***modified*** DNA. The amplification ***products*** are resolved on native polyacrylamide gels and visualized by staining with ethidium bromide followed by UV illumination. Amplification products detected. .
- DETD . . . islets compared with various other mouse tissues (Lenzen 1996.sup.548). Moreover, induction of cellular stress by high glucose, high oxygen, and ***heat*** ***shock*** treatment did not affect antioxidant enzyme expression in rat pancreatic islets or in RINm5F insulin-producing cells (Tiedge 1997.sup.549). Based on. . .
- DETD . . . copy number of viral genome in the subsequent latent period. Infection with some viruses, such as measles, hepatitis A, and ***Mycobacterium*** tuberculosis induce a strong polarized Th1-type response in early life. These infections reduce GABP virus replication and subsequent genome copy. . .
- DETD [2107] BCG is a freeze-dried preparation made from a living culture of the Calmette-Guerin strain of ***mycobacterium*** Bovis. It was first developed as a vaccine against tuberculosis in 1921 but also has been used as an immunotherapeutic. . .
- DETD [2108] Results of numerous studies suggest that measles, hepatitis A, and ***Mycobacterium*** tuberculosis infection in early life may prevent subsequent development of atopic diseases. In humans, immunomodulation during the first two years. . .
- DETD [2110] Another study showed that an infection of NOD mice with ***Mycobacterium*** avium, before the mice show overt diabetes, results in permanent protection of the animals from diabetes. This protective effect was. . .
- DETD . . . V V, Nakatani Y, Wolffe A P. Xenopus NF-Y pre-sets chromatin to potentiate p300 and acetylation-responsive transcription from the Xenopus ***hsp70*** promoter in vivo. EMBO J. 1998 Nov 2;17(21):6300-15.
- DETD . . . S, Wheeler J, Huygen K, Aaby P, McAdam K P, Newport M J. Newborns develop a Th1-type immune response to ***Mycobacterium*** bovis bacillus Calmette-Guerin vaccination. J Immunol. 1999 Aug 15;163(4):2249-55.
- DETD [2897] .sup.780 von Hertzen L, Klaukka T, Mattila H, Haahtela T. ***Mycobacterium*** tuberculosis infection and the subsequent development of asthma and allergic conditions. J Allergy Clin Immunol. 1999 Dec; 104(6):1211-4.
- DETD [2903] .sup.786 Martins T C, Aguas A P. Mechanisms of ***Mycobacterium*** avium-induced resistance against insulin-dependent diabetes mellitus (IDDM) in non-obese diabetic (NOD) mice: role of Fas and Th1 cells. Clin Exp. . .
- DETD [2904] .sup.787 Bras A, Aguas A P. Diabetes-prone NOD mice are resistant to ***Mycobacterium*** avium and the infection prevents autoimmune disease. Immunology. 1996 Sep;89(1):20-5.

L12 ANSWER 7 OF 14 USPATFULL on STN
AN 2003:99511 USPATFULL
TI Drug discovery assays based on microcompetition for a limiting GABP complex
IN Polansky, Hanan, Rochester, NY, UNITED STATES
PI US 2003068616 A1 20030410
AI US 2002-223050 A1 20020814 (10)
RLI Continuation-in-part of Ser. No. US 2000-732360, filed on 7 Dec 2000, PENDING
DT Utility
FS APPLICATION
LREP Hanan Polansky, 3159 S. Winton Rd., Rochester, NY, 14623
CLMN Number of Claims: 55
ECL Exemplary Claim: 1
DRWN 28 Drawing Page(s)
LN.CNT 14981

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A recent discovery showed that microcompetition for GABP between a foreign polynucleotide and a cellular GABP regulated gene is a risk factor for some of the major chronic diseases, such as obesity, cancer, atherosclerosis, stroke, osteoarthritis, diabetes, asthma, and other autoimmune diseases. The invention uses this novel discovery to present assays for screening compounds based on their effectiveness in modulating such microcompetition, or the effects of such microcompetition on the cell. The selected compounds can be used in treatment of these chronic diseases. The invention also presents assays for screening compounds that can be used in treatment of chronic diseases resulting from other foreign polynucleotide-type disruptions.

DETD . . . ExoKII, FAS, TSP-1, FGF-4, .alpha.1-chim, Tr Hydr, NaKATPsea-3, PDFG.beta., FerH, MHC IA2 B8, Cw2Ld and B7, MDR1, CYP1A1, c-JUN, Grp78, ***Hsp70***, ADH2, GPAT, FPP, HMG, HSS, SREBP2, GHR, CP2, .beta.-actin, TK, TopoII.alpha., I, III, III, IV, cdc25, cdc2, cyclA, cyclB1, E2F1, . . .

DETD . . . DNA sample, one using primers specific for the DNA prior to bisulfite treatment, and one using primers for the chemically ***modified*** DNA. The amplification ***products*** are resolved on native polyacrylamide gels and visualized by staining with ethidium bromide followed by UV illumination. Amplification products detected. .

DETD . . . islets compared with various other mouse tissues (Lenzen 1996.sup.548). Moreover, induction of cellular stress by high glucose, high oxygen, and ***heat*** ***shock*** treatment did not affect antioxidant enzyme expression in rat pancreatic islets or in RINm5F insulin-producing cells (Tiedge 1997.sup.549). Based on. . .

DETD . . . copy number of viral genome in the subsequent latent period. Infection with some viruses, such as measles, hepatitis A, and ***Mycobacterium*** tuberculosis induce a strong polarized Th1-type response in early life. These infections reduce GABP virus replication and subsequent genome copy. . .

DETD [2087] BCG is a freeze-dried preparation made from a living culture of the Calmette-Guerin strain of ***mycobacterium*** Bovis. It was first developed as a vaccine against tuberculosis in 1921 but also has been used as an immunotherapeutic. . .

DETD [2088] Results of numerous studies suggest that measles, hepatitis A, and ***Mycobacterium*** tuberculosis infection in early life may prevent subsequent development of atopic diseases. In humans, immunomodulation during the first two years. . .

DETD [2090] Another study showed that an infection of NOD mice with
 Mycobacterium avium, before the mice show overt diabetes,
 results in permanent protection of the animals from diabetes. This
 protective effect was. . .

DETD . . . V V, Nakatani Y, Wolffe A P. Xenopus NF-Y pre-sets chromatin to
 potentiate p300 and acetylation-responsive transcription from the
 Xenopus ***hsp70*** promoter in vivo. EMBO J. Nov. 2,
 1998;17(21):6300-15.

DETD . . . Wheeler J, Huygen K, Aaby P, McAdam K P, Newport M J. Newborns
 develop a Th 1-type immune response to ***Mycobacterium*** bovis
 bacillus Calmette-Guerin vaccination. J. Immunol. Aug. 15,
 1999;163(4):2249-55.

DETD [2877] .sup.780 von Hertzen L, Klaukka T, Mattila H, Haahtela T.
 Mycobacterium tuberculosis infection and the subsequent
 development of asthma and allergic conditions. J Allergy Clin Immunol.
 1999 December;104(6):1211-4.

DETD [2883] .sup.786 Martins T C, Aguas A P. Mechanisms of
 Mycobacterium avium-induced resistance against insulin-
 dependent
 diabetes mellitus (IDDM) in non-obese diabetic (NOD) mice: role of Fas
 and Th1 cells. Clin Exp. . . .

DETD [2884] .sup.787 Bras A, Aguas A P. Diabetes-prone NOD mice are resistant
 to ***Mycobacterium*** avium and the infection prevents autoimmune
 disease. Immunology. 1996 September;89(1):20-5.

L12 ANSWER 8 OF 14 USPATFULL on STN

AN 2003:40533 USPATFULL

TI Methods for the inhibition of epstein-barr virus transmission employing
 anti-viral peptides capable of abrogating viral fusion and transmission

IN Barney, Shawn O'Lin, Cary, NC, United States

Lambert, Dennis Michael, Cary, NC, United States

Petteway, Stephen Robert, Cary, NC, United States

PA Trimeris, Inc., Durham, NC, United States (U.S. corporation)

PI US 6518013 B1 20030211

AI US 1995-485546 19950607 (8)

RLI Continuation-in-part of Ser. No. US 1994-360107, filed on 20 Dec 1994,
 now patented, Pat. No. US 6017536 Continuation-in-part of Ser. No. US
 1994-255208, filed on 7 Jun 1994 Continuation-in-part of Ser. No. US
 1993-73028, filed on 7 Jun 1993, now patented, Pat. No. US 5464933

DT Utility

FS GRANTED

EXNAM Primary Examiner: Scheiner, Laurie; Assistant Examiner: Parkin, Jeffrey
 S.

LREP Pennie & Edmonds LLP, Nelson, M. Bud

CLMN Number of Claims: 22

ECL Exemplary Claim: 1

DRWN 84 Drawing Figure(s); 83 Drawing Page(s)

LN.CNT 24700

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Fusion of the viral envelope, or infected cell membranes with uninfected
 cell membranes, is an essential step in the viral life cycle. Recent
 studies involving the human immunodeficiency virus type 1(HIV-1)
 demonstrated that synthetic peptides (designated DP-107 and DP-178)
 derived from potential helical regions of the transmembrane (TM)
 protein, gp41, were potent inhibitors of viral fusion and infection. A
 computerized antiviral searching technology (C.A.S.T.) that detects
 related structural motifs (e.g., ALLMOTI 5, 107.times.178.times.4, and

PLZIP) in other viral proteins was employed to identify similar regions in the Epstein-Barr virus (EBV). Several conserved heptad repeat domains that are predicted to form coiled-coil structures with antiviral activity were identified in the EBV genome. Synthetic peptides of 16 to 39 amino acids derived from these regions were prepared and their antiviral activities assessed in a suitable in vitro screening assay. These peptides proved to be potent inhibitors of EBV fusion. Based upon their structural and functional equivalence to the known HIV-1 inhibitors DP-107 and DP-178, these peptides should provide a novel approach to the development of targeted therapies for the treatment of EBV infections.

DETD . . . HAEMOPHILUS DUCREYI 339-366 417-444
PCH60_LEGMI 60 KD CHAPERONIN LEGIONELLA MICDADEI 299-333
PCH60_LEGPN 60 KD CHAPERONIN LEGIONELLA PNEUMOPHILA 298-332 452-479
PCH60_MYCLE 60 KD CHAPERONIN ***MYCOBACTERIUM*** LEPRAE 125-152 236-263
337-364
PCH60_MYCTU 60 KD CHAPERONIN ***MYCOBACTERIUM*** TUBERCULOSIS 125-152
337-364
& BOVIS
PCH60_PSEAE 60 KD CHAPERONIN PSEUDOMONAS AERUGINOSA 339-366
PCH60_RHILV 60 KD CHAPERONIN RHIZOBIUM LEGUMINOSARUM 117-163 322-370 425-466
PCH60_RICTS 60. . . CAULOBACTER CRESCENTUS 561-588
PDNAK_CLOAB DNAK PROTEIN CLOSTRIDIUM ACETOBUTYLICUM 499-526
PDNAK_CLOPE DNAK PROTEIN CLOSTRIDIUM PERFRINGENS 496-527
PDNAK_METMA DNAK PROTEIN METHANOSARCINA MAZEI 523-550
PDNAK_MYCTU DNAK PROTEIN ***MYCOBACTERIUM*** TUBERCULOSIS 502-529
PDNAK_STRCO DNAK PROTEIN STREPTOMYCES COELICOLOR 45-72 533-572
PDNIR_ECOLI REGULATORY PROTEIN DNIR ESCHERICHIA COLI 114-141
PDNLI_ZYMMO DNA LIGASE ZYMOMONAS MOBILIS 658-712
PDNRJ_STRPE TRANSDUCTION. . .
DETD . . . THERMOPLASMA ACIDOPHILUM 13-40 49-76 220-247
PEFG_ANANI ELONGATION FACTOR G ANACYSTIS NIDULANS 332-359
PEFG_ECOLI ELONGATION FACTOR G ESCHERICHIA COLI 234-261
PEFG_MYCLE ELONGATION FACTOR G ***MYCOBACTERIUM*** LEPRAE 211-259 330-357
PEFG_SALTY ELONGATION FACTOR G SALMONELLA TYPHIMURIUM 234-261
PEFG_SPIPL ELONGATION FACTOR G SPIRULINA PLATENSIS 334-374 481-511
PEFG_SYNY3 ELONGATION FACTOR G SYNECHOCYSTIS. . . FACTOR TU HALOARcula
MARISMORTUI 4-31
PEFTU_MICLU ELONGATION FACTOR TU MICROCOCCUS LUTEUS 221-248
PEFTU_MYCHO ELONGATION FACTOR TU MYCOPLASMA HOMINIS 222-249
PEFTU_MYCLE ELONGATION FACTOR TU ***MYCOBACTERIUM*** LEPRAE 220-257
PEFTU_MYCTU ELONGATION FACTOR TU ***MYCOBACTERIUM*** TUBERCULOSIS 220-247
PEFTU_SHEPU ELONGATION FACTOR TU SHEWANELLA PUTREFACIENS 26-53
PEFTU_STORR ELONGATION FACTOR TU STREPTOCOCCUS ORALIS 232-259
PELAS_PSEAE PSEUDOLYSIN PRECURSOR PSEUDOMONAS AERUGINOSA 141-168
PELT1_ECOLI T-LABILE. . .
DETD . . . TYPHIMURIUM 8-35
MEMBRANE Q PROTEIN
PHISX_ECOLI HISTIDINOL DEHYDRO- ESCHERICHIA COLI 393-434
GENASE
PHISX_LACLA HISTIDINOL DEHYDRO- LACTOCOCCUS LACTIS 19-46 264-303
GENASE
PHISX_MYCSM HISTIDINOL DEHYDRO- ***MYCOBACTERIUM*** SMEGMATIS 288-329
399-430
GENASE
PHISX_SALTY HISTIDINOL DEHYDRO- SALMONELLA TYPHIMURIUM 393-434
GENASE

PHLA_STAAU ALPHA-HEMOLYSIN STAPHYLOCOCCUS AUREUS 69-102
 PRECURSOR
 PHLY1_ECOLI HEMOLYSIN A, CHROMO- ESCHERICHIA COLI. . . SOLANACEARUM 371-405
 PHRP_H_PSESY OUTER MEMBRANE PROTEIN PSEUDOMONAS SYRINGAE 102-129 310-344
 HRP_H PRECURSOR
 PHRPS_PSESH PROBABLE REGULATORY PSEUDOMONAS SYRINGAE 24-51
 PROTEIN HRP_S
 PHS18_CLOAB 18 KB ***HEAT*** ***SHOCK*** PROTEIN CLOSTRIDIUM
 ACETOBUTYLICUM 67-108
 PHS70_HALMA ***HEAT*** ***SHOCK*** 70 KD PROTEIN HALOARcula MARISMORTUI
 522-576
 PHS70_MYCLE ***HEAT*** ***SHOCK*** 70 KD PROTEIN ***MYCOBACTERIUM***
 LEPRAE 461-488 503-530
 PHS70_MYCPA ***HEAT*** ***SHOCK*** 70 KD PROTEIN ***MYCOBACTERIUM***
 PARA- 460-487
 TUBERCULOSIS
 PHTPG_ECOLI ***HEAT*** ***SHOCK*** PROTEIN C62.5 ESCHERICHIA COLI
 221-248 482-509
 PHTRA_ECOLI PROTEASE DO PRECURSOR ESCHERICHIA COLI 373-400
 PHTRE_ECOLI HTRE PROTEIN PRECURSOR ESCHERICHIA COLI 454-484 524-576
 PHTRJ_HALHA SENSORY. . .
 DETD . . . 135-162 232-269 288-315
 PRECA_METCL RECA PROTEIN METHYLOMONAS CLARA 266-303
 PRECA_METFL RECA PROTEIN METHYLOBACILLUS FLAGELLATUM 276-303
 PRECA_MYCPU RECA PROTEIN MYCOPLASMA PULMONIS 30-57
 PRECA_MYCTU RECA PROTEIN ***MYCOBACTERIUM*** TUBERCULOSIS 749-776
 PRECA_NEIGO RECA PROTEIN NEISSERIA GONORRHOEAE 263-310
 PRECA_PROMI RECA PROTEIN PROTEUS MIRABILIS 283-310
 PRECA_PSEAE RECA PROTEIN PSEUDOMONAS AERUGINOSA 282-309
 PRECA_RHILP RECA PROTEIN RHIZOBIUM. . . A
 PRPOA_THECE DNA-DIRECTED RNA THERMOCOCCUS CELER 228-262
 POLYMERASE SUBUNIT A'
 PRPOB_ECOLI DNA-DIRECTED RNA ESCHERICHIA COLI 599-626 1011-1038
 POLYMERASE BETA CHAIN
 PRPOB_MYCLE DNA-DIRECTED RNA ***MYCOBACTERIUM*** LEPRAE 723-760 1084-1111
 POLYMERASE BETA CHAIN
 PRPOB_SALTY A-DIRECTED RNA SALMONELLA TYPHIMURIUM 599-626 958-985 1011-1038
 POLYMERASE BETA CHAIN
 PRPOB_SULAC A-DIRECTED RNA SULFOLOBUS ACIDOCALDARIUS. . . HALOCOCCUS
 MORRHUAE 27-54 117-144 207-234
 POLYMERASE SUBUNIT C
 PRPOC_METTH DNA-DIRECTED RNA METHANOBACTERIUM 58-85 272-302 327-354
 POLYMERASE SUBUNIT C THERMOAUTOTROPHICU
 PRPOC_MYCLE A-DIRECTED RNA ***MYCOBACTERIUM*** LEPRAE 273-300 860-887
 911-938 1131-1158
 POLYMERASE BETA' CHAIN
 PRPOC_NOSCO DNA-DIRECTED RNA NOSTOC COMMUNE 150-192
 POLYMERASE GAMMA CHAIN
 PRPOC_SULAC DNA-DIRECTED RNA SULFOLOBUS ACIDOCALDARIUS. . . 35-62 182-216
 PRS6_THETH 30S RIBOSOMAL PROTEIN S6 THERMUS AQUATICUS 16-43
 PRS7_METVA 30S RIBOSOMAL PROTEIN S7 METHANOCOCCUS VANNIELII 69-96
 PRS7_MYCLE 30S RIBOSOMAL PROTEIN S7 ***MYCOBACTERIUM*** LEPRAE 22-49
 PRS8_MICLU 30S RIBOSOMAL PROTEIN S8 MICROCOCCUS LUTEUS 103-130
 PRS8_MYCCA 30S RIBOSOMAL PROTEIN S8 MYCOPLASMA CAPRICOLUM 41-78
 PRSGA_ECOLI FERRITIN LIKE PROTEIN ESCHERICHIA. . .
 DETD . . . COLI 181-208 308-340 720-754
 PTR66_ECOLI TRANSPOSASE ESCHERICHIA COLI 51-78

PTRAC6_SHISO TRANSPOSASE SHIGELLA SONNEI 51-78 200-227 231-258
 PTRAC7_ECOLI TRANSPOSASE ESCHERICHIA COLI 729-756
 PTRAC9_MYCTU PUTATIVE TRANSPOSASE ***MYCOBACTERIUM*** TUBERCULOSIS 159-186
 PTRAB_BACTB IS231B PROBABLE BACILLUS THURINGIENSIS 281-308 419-446
 TRANSPOSASE
 PTRAC_BACTB IS231C PROBABLE BACILLUS THURINGIENSIS 281-308 419-446
 TRANSPOSASE
 PTRAC_STAAU TRANSPOSASE STAPHYLOCOCCUS AUREUS 4-31. . .
 DETD . . . HYPOTHETICAL PROTEIN ESCHERICHIA FERGUSONII 2-35
 PYAM1_SALTY PUTATIVE AMIDASE SALMONELLA TYPHIMURIUM 73-100
 PYAT1_SYNY3 HYPOTHETICAL 13.0 KD SYNECHOCYSTIS SP 26-60
 PROTEIN
 PYATP_MYCLE HYPO PROTEIN PUTATIVE ***MYCOBACTERIUM*** LEPRAE 23-57 91-158
 511-538
 ATP OPERON
 PYATR_BACFI HYPOL ATP-BINDING BACILLUS FIRMUS 211-238
 TRANSPORT PROTEIN
 PYATS_MYCGA HYPOTHETICAL PROTEIN MYCOPLASMA GALLISEPTICUM 7-41
 PYATU_MYCGA HYPOTHETICAL PROTEIN. . . LACTOBACILLUS HELVETICUS 93-120
 127-154
 PYHYA_PSESN HYPOTHETICAL PROTEIN PSEUDOMONAS SP 217-266
 PYI11_HALHA HYPOTHETICAL 38.0 KD HALOBACTERIUM HALOBIIUM 245-272
 PROTEIN
 PYI32_MYCTU IS986 HYPOTHETICAL 6.6 KD ***MYCOBACTERIUM*** TUBERCULOSIS
 19-46
 PROTEIN
 PYI42_PSEAY HYPOTHETICAL 42.6 KD PSEUDOMONAS AMYLODERAMOSA 9-36
 PROTEIN
 PYI48_METSM ISM1 HYPOTHETICAL 48.3 KD METHANOBREVIBACTER SMITHII 73-100
 154-184 338-365
 PROTEIN
 PYI52_HALHA. . .
 DETD . . . 102-129
 (ACIDIC FIBROBLAST
 PHBG3_HUMAN INT-2 PROTO-ONCOGENE PROTEIN PRECURSOR (HBGF-3) 61-91
 PHBG6_HUMAN FIBROBLAST GROWTH FACTOR-6 PRECURSOR (FGF-6) 41-75 159-186
 (HBGF-6) (HST-2)
 PHBI_HUMAN P59 PROTEIN (***HSP*** BINDING IMMUNOPHILIN) (HBI) 264-312
 (POSSIBLE PEPTIDYL-PROLYL
 PHEM4_HUMAN UROPORPHYRINOGEN-III SYNTHASE (EC 4.2.1.75) 74-118
 (UROPORPHYRINOGEN-III
 PHEP2_HUMAN HEPARIN COFACTOR II PRECURSOR (HC-II) (PROTEASE 169-196
 INHIBITOR. . . 1.13.11.27) 306-333
 (4HPPD)
 PHRX_HUMAN ZINC FINGER PROTEIN HRX 521-548 914-974 1637-1666 2215-2286
 2289-2316 3317-3344 3448-3475
 PHS1_HUMAN HEMATOPOIETIC LINEAGE CELL SPECIFIC PROTEIN 43-70
 PHS9A_HUMAN ***HEAT*** ***SHOCK*** PROTEIN ***HSP*** 90-ALPHA (***HSP***
 86) 443-470 640-674
 PHSER_HUMAN HEAT-STABLE ENTEROTOXIN RECEPTOR PRECURSOR (GC-C) 511-545
 (INTESTINAL
 PHSF1_HUMAN ***HEAT*** ***SHOCK*** FACTOR PROTEIN 1 (HSF 1) (***HEAT***
 SHOCK 113-140 168-209
 TRANSCRIPTION FACTOR
 PHSF2_HUMAN ***HEAT*** ***SHOCK*** FACTOR PROTEIN 2 (HSF 2) (***HEAT***
 SHOCK 117-198
 TRANSCRIPTION FACTOR

PHV2I_HUMAN IG HEAVY CHAIN PRECURSOR V-II REGION (ARH-77) 67-108
PHV3T_HUMAN IG HEAVY CHAIN V-III REGION (GAL) 47-74
PHX11_HUMAN HOMEBOX PROTEIN. . .
DETD . . . Leu Asp Lys Tyr

20 25 30

Lys Asn Ala

35

SEQUENCE CHARACTERISTICS:

LENGTH: 35 amino acids

TYPE: amino acid

STRANDEDNESS:

TOPOLOGY: unknown

MOLECULE TYPE: peptide

FEATURE:

NAME/KEY: ***Modified*** -site

OTHER INFORMATION: / ***product*** = "OTHER"
represents U, the standard designation
modified cysteine."

/note= "X
for C-abu, a

SEQUENCE: 127

Ser Asn Ile Lys Glu Asn Lys. . . Val Thr Glu Leu

20 25 30

Gln Leu Leu

35

SEQUENCE CHARACTERISTICS:

LENGTH: 35 amino acids

TYPE: amino acid

STRANDEDNESS:

TOPOLOGY: unknown

MOLECULE TYPE: peptide

FEATURE:

NAME/KEY: ***Modified*** -site

OTHER INFORMATION: / ***product*** = "OTHER"
represents U, the standard designation
modified cysteine."

/note= "X
for C-abu, a

SEQUENCE: 128

Lys Glu Asn Lys Xaa Asn Gly. . .

L12 ANSWER 9 OF 14 USPATFULL on STN

AN 2002:315069 USPATFULL

TI Compositions and methods for treatment of neoplastic disease

IN Terman, David S., Pebble Beach, CA, UNITED STATES

PI US 2002177551 A1 20021128

AI US 2001-870759 A1 20010530 (9)

PRAI US 2000-208128P 20000531 (60)

DT Utility

FS APPLICATION

LREP David S. Terman, P.O. Box 987, Pebble Beach, CA, 93953

CLMN Number of Claims: 30

ECL Exemplary Claim: 1

DRWN 3 Drawing Page(s)

LN.CNT 17323

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention comprises compositions and methods for treating a tumor or neoplastic disease in a host, The methods employ conjugates comprising superantigen polypeptides, nucleic acids with other structures that preferentially bind to tumor cells and are capable of inducing apoptosis. Also provided are superantigen-glycolipid conjugates

and vesicles that are loaded onto antigen presenting cells to activate both T cells and NKT cells. Cell-based vaccines comprise tumor cells engineered to express a superantigen along with glycolipids products which, when expressed, render the cells capable of eliciting an effective anti-tumor immune response in a mammal into which these cells are introduced. Included among these compositions are tumor cells, hybrid cells of tumor cells and accessory cells, preferably dendritic cells. Also provided are tumoricidal T cells and NKT cells devoid of inhibitory receptors or inhibitory signaling motifs which are hyperresponsive to the the above compositions and lipid-based tumor associated antigens that can be administered for adoptive immunotherapy of cancer and infectious diseases.

SUMM . . . cell both enterotoxins and .alpha.-galactosylceramides are associated with numerous intracellular and membrane structures such as MHC, costimulatory and adhesion molecules, ***heat*** ***shock*** proteins, membrane glycolipids and glycosphingolipids which may improve immunogenicity and antigen presentation. They may also be transported in various vesicles. . . .

SUMM . . . structures may actually improve the T cell activating function of SAGs such as deoxyribonucleic acids, ribonucleic acids, tumor associated antigens, ***heat*** ***shock*** proteins, costimulatory molecules and adhesion molecules and endosomes. Cellular SAG peptides or nucleotides exist in association with tumor associated antigens, costimulants, adhesion molecules, ***heat*** ***shock*** proteins and MHC molecules, GPI-ceramides or SAG receptors (digalactosylceramides) which improve the immunogenicity of the tumor antigens. Therefore, these structural. . . .

SUMM . . . tumor cells, an immunogenic bacterial product such as Staphylococcal adhesin protein A, LPS, .beta.-glucans, and peptidoglycans, costimulatory and adhesion molecules, ***heat*** ***shock*** protein, growth factor receptors such as Her/neu and tumor markers such as PSA.

DRWD . . . Oncogenes, amplified oncogenes and transcription factors

15. Angiogenic factors and receptors

16. Tumor growth factor receptors

17. Tumor suppressor receptors

18. Cell cycle proteins

19. ***Heat*** - ***shock*** proteins, ATPases and G proteins

20. Proteins engaged in antigen processing, sorting and intracellular trafficking

21. Inducible nitric oxide synthase (iNOS)

22. apolipoproteins. . . .

DETD . . . the ability to stimulate large subsets of T cells. SAGs include Staphylococcal enterotoxins, Streptococcal pyrogenic exotoxins, mycoplasma antigens, rabies antigens, ***mycobacteria*** antigens, EB viral antigens, minor lymphocyte stimulating antigen, mammary tumor virus antigen, ***heat*** ***shock*** proteins, stress peptides, clostridial and toxoplasmosis antigens and the like. Any SAG can be used as described herein, although, Staphylococcal. . . .

DETD . . . in gram positive bacteria (such as Staphylococcus or Streptococcus), to nucleic acids encoding capsular polysaccharides and teichoic acids and in ***mycobacterial*** species to nucleic acids encoding lipoarabinan.

DETD . . . E. coli, Salmonella or Klebsiella or for bacteria which naturally produce lipoarabinomannans glycans or polysaccharides containing cell walls such as ***mycobacterium*** and streptococcus

respectively. The SAg-polysaccharide constructs bind to CD1 receptors of antigen presenting cells. They are then capable of activating. . .

DETD . . . J et al., J. Natl. Cancer Inst. 72:955-962 (1984)). By synthesizing single stranded nucleotides corresponding to different regions in the ***Mycobacterium*** bovis genome, specific single stranded oligonucleotides that activate adherent splenocytes and enhanced natural killer cell activity have been identified. In. . .

DETD [0218] Examples are LPS's of gram negative organisms, SAGs and peptidoglycans of gram positive organisms, fungal .beta.-glucans, bacterial glycosylceramides, and ***mycobacterial*** lipoarabinans. Numerous infectious agents with these structures cause potent immune reactions e.g. streptococcal cellulitis induced by S. pyogenes, E. coli.

DETD . . . molecule interact with different types of host cells. There is also evidence that immunopotentiating activity of a glycopeptide produced by ***mycobacteria*** is dependent on the saccharide residues of the molecule.

DETD [0249] Genes Involved in ***Mycobacterial*** Cell Wall Biosynthesis

DETD . . . fused in frame or cotransfected into tumor cell with nucleic acids encoding the key enzymes involved in the biosynthesis of ***mycobacterial*** cell wall mycolic acid, phosphatidylinositol mannosides and lipoarabinans. A high affinity interaction of CD1b molecules with the acyl side chains. . .

DETD . . . in the biosynthesis of these molecules have been isolated. In addition to the usual fatty acids found in membrane lipids, ***mycobacteria*** have a wide variety of very long-chain saturated (C18-C32) and monounsaturated (up to C26) n-fatty acids. The occurrence of a-alkyl b-hydroxy very long chain fatty acids i.e., mycolic acid is a hallmark of ***mycobacteria*** and related species.

Mycobacterial mycolic acids are the largest (C70-C90) with the largest -branch (C20-C25). The main chain contains one or two double bonds,. . . esterified to glycerol or sugars such as trehalose, glucose and fructose depending on the sugars present in the culture medium. ***Mycobacterium*** also contains several methyl-branched fatty acids. These include 10-methyl C18 fatty acid (tuberculoheptanoic acid found esterified in phosphatidyl inositide mannosides),. . .

DETD [0255] The MAS gene encoding ***mycobacterial*** mycoerolic acid synthase is a dimer of the FAS gene. The cloning and sequencing of the MAS gene revealed the. . .

DETD . . . results in tissue specific forms of the protein, which can be intracellular, membrane bound, or secreted. In cells infected with ***mycobacteria***, the CD1 molecule binds and presents a ***mycobacterial*** membrane component, mycolic acid. Surface CD1 molecules present longer peptides than those normally found on class I molecules. Whether CD1. . .

DETD . . . Nucleic acid encoding cell wall or cell membrane associated glycosylceramides or a branched, b hydroxy long-chain fatty acids found in ***mycobacteria*** and other bacteria are cotransfected into the CD1 transfected tumor cells. The tumor cell therefore displays glycosylceramides bound to the. . .

DETD [0366] 36. SAGs Combined with Signal Transduction Molecules or ***Heat*** ***Shock*** Proteins (***HSPs***)

DETD . . . frame to (or cotransfected with) a nucleic acid encoding "signal transduction molecules" such as Ras, JAK 1 and STAT-1a and ***heat*** ***shock*** proteins ***HSP*** -60, ***HSP*** -70, ***HSP*** -90a, ***HSP*** -90b, Cox-2 as well as heterotrimeric G proteins and ATPases. The genes for Staphylococcal

HSP -70 (SEQ ID NOS:42-43) useful in this invention have been cloned (Ohta, T et al., J. Bacteriology 176: 4779-4783, (1994)). As . . of above structures at the peptide or nucleic acid level. Preferred proteins for this embodiment are G proteins, ATPases and ***HSPs*** . Chemical conjugation is carried out by conventional methods, e.g., use of preferred heterobifunctional crosslinkers. Alternatively, conjugates are produced genetically as. . .

DETD [0368] SAg-encoding nucleic acid is fused in frame (or cotransfected) with nucleic acid encoding a signal transduction protein or ***HSP*** . Transfectants are prepared as in Example 1. They are used in vivo as a preventative or therapeutic antitumor vaccine according. . .

DETD . . . 37. SAGs with Specialized Sites for C-terminal GPI Anchoring, Glycosylation Sulfation, N-Myristoylation, Phosphorylation, Hydroxylation, N-Methylation, Signal Peptide Binding, LPS Binding, ***HSP*** Binding, Chemokine Binding and Prenylation

DETD [0375] Nucleic acids encoding ***HSPs*** , along with their promoters, are fused in-frame (or cotransfected) with SAG nucleic acid. These include but are not limited to two recently discovered ***HSP*** genes, orf37 and orf35 in Staphylococcus aureus that are upstream and downstream of grpE (***hsp20***), dnaK (***hsp70***) and dnaJ (***hsp40***) in the following sequence: orf37- ***hsp20*** - ***hsp70*** - ***hsp40*** -orf35. The promoters are located upstream of orf37 and upstream of ***hsp40*** . These fused proteins are useful as preventative or therapeutic antitumor vaccines according to Examples 15, 16, 18-23. They are also. . .

DETD . . . (in terms of number) SAG receptor site binds to tumor cells expressing SAG receptors. SAGs possess a site for binding ***HSPs*** which are useful in immunizing normal or anergic T cells in a tumor patient. SAGs bind to T cell antagonist. . .

DETD . . . Furthermore, nucleic acids encoding proteins listed in Tables I, II, IV and V, for example, angiostatin, protein A, erb/Neu and ***HSPs*** , staphylococcal collagen adhesin, are introduced into and expressed in tumor cells or DCs that express or secrete SAG, or into. . .

DETD . . . pathway. Owing to the lower B-carotene content, Lp(a) may be more easily oxidized than LDL. Oxidized Lp(a) such as Lp(a) ***modified*** by malondialdehyde, a ***product*** generated in vivo from aggregated platelets, is avidly taken up by monocyte-macrophages, through the scavenger-receptor pathway. Lp(a) accumulates in either. . .

DETD [0589] IR.sub.IDAs recognize Lip-IDAs derived from bacteria, ***mycobacteria*** , parasites, fungi, protozoans or plants and respond by producing an inhibitory T cell response. Lip-IDAs comprise sphingolipids, glycopeptides, phytoglycolipids, mycoglycolipids,. . .

DETD . . . types listed above). In another embodiment, SAGs are conjugated to Lip-IDAs such as glycans and peptidoglycan antigens derived from bacteria, ***mycobacteria*** , parasites, fungi or plants. These families are listed above. These lipid based molecules also include sphingolipids with inositolphosphate-containing head groups. . .

DETD [0614] Conjugates between SAG and a Lip-IDA derived from a fungal, parasitic or ***mycobacterial*** sources are also used for the treatment of infectious diseases such as tuberculosis, leishmaniasis, trypanosomiasis as disclosed in Example 53.. . . are also useful ex vivo for activating a population of cells in which IR.sub.IDAs specific for bacterial, fungal, parasitic or ***mycobacterial*** antigens have been (1) deleted (via gene knockout) or (2) functionally

inactivated (via antisense) for use in adoptive immunotherapy of. . .

DETD . . . 92: 1619-1623
(1995)

31. Lipid A biosynthetic (SEQ ID NOS:105-112) Tumor
Schnaitman CA et al.,
genes lpxA-D
Microbiological
Reviews 57: 655-682
(1993)

32. ***Mycobacterial*** mycolic acid (SEQ ID NOS:113-114) Tumor
Fernandes ND et al.,
Gene 170: 95-99 (1996);
Mathur M et al., J.Biol.
Chem.. . .

DETD . . . and greatly restricted anchors are preferred. This recognition
of CD1-presented antigens depends on the type and distribution of sugar
residues. ***Mycobacterial*** cell wall antigens namely mycolic
acids and lipoarabinomannan also bind to CD1. Recently several
glycosylceramides, in particular, monogalactosyl ceramides GalCer). .

DETD . . . or exosomes comprising SAg-GalCer complexes or SAg-tumor
peptide (including but not limited to normal mutated structures). The
ternary complexes of SAg-GalCer- ***heat*** ***shock*** protein
and tumor peptide- ***heat*** ***shock*** protein are also
useful, These complexes may be in or soluble or immobilized form,
attached to a CD1 or MHC. . .

DETD . . . acid and growth factor receptor nucleic acids18. SAg-encoding
nucleic acid and cell cycle protein nucleic acids

19. SAg-encoding nucleic acid and ***heat*** ***shock*** protein
nucleic acids

20. SAg-encoding nucleic acid and chemokine nucleic acids

21. SAg-encoding nucleic acid and cytokine nucleic acids

22. SAg-encoding nucleic acid. . .

DETD . . . molecules containing amino acid sequences and homologous to the
enterotoxin family of molecules. To this extent, mammary tumor virus
sequences, ***heat*** ***shock*** proteins, stress peptides,
Mycoplasma and ***mycobacterial*** antigens, and minor lymphocyte
stimulating loci bearing tumoricidal structural homology to the
enterotoxin family are useful as anti-tumor agents. Hybrid. . .

DETD . . . commonly used inducible promoters include the metallothionein
CUPI promoter, which is tightly controlled by copper; promoters
activated in response to ***heat*** ***shock*** , which are of
particular interest for expression in the temperature-sensitive sec6-4
mutant and the PH05 promoter, which is derepressed at. . .

DETD . . . you using beige mutants on a B6 background? This is not clear
here are infected with many of the nontuberculous ***mycobacteria***
: MAC (what is this), M. kansasii, M. simiac, M. malmoense or M.
genavense. Same-sex mice aged 5-7 weeks are allowed. . .

DETD [1853] Primary cultures of MAC (M. kansasii or other
mycobacteria) to be used for infection are obtained from
clinical isolates of patients with disseminated MAC infection, or the
American Type. . .

DETD . . . bacilli in the lungs the infection grows progressively at first
and is then curtailed around 20 days. Laboratory strains of
mycobacteria such as Erdman attain 4-5 logs in the lungs by
this
time. More virulent strains such as CSU93 (Tennessee outbreak). . .

DETD [1918] Preparation of Lip-TAAs and Lip-IDAs of Bacterial, Fungal, Yeast, Parasitic, ***Mycobacterial***, Invertebrate or Protozoan Origin

L12 ANSWER 10 OF 14 USPATFULL on STN

AN 2002:307566 USPATFULL

TI Methods and compositions for therapeutic intervention in infectious disease

IN Stewart, Graham, Walton-on-Thames, UNITED KINGDOM

O'Gaora, Peadar, London, UNITED KINGDOM

Young, Douglas, Ruislip, UNITED KINGDOM

PI US 2002172685 A1 20021121

AI US 2002-79136 A1 20020220 (10)

PRAI US 2001-269801P 20010220 (60)

US 2001-294170P 20010529 (60)

DT Utility

FS APPLICATION

LREP JOHN S. PRATT, ESQ, KILPATRICK STOCKTON, LLP, 1100 PEACHTREE STREET, SUITE 2800, ATLANTA, GA, 30309

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 15 Drawing Page(s)

LN.CNT 1922

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods and compositions for the treatment and prevention of infectious diseases are provided. In particular, efficient vaccines comprising genetically modified pathogens are provided. The vaccines generally comprise ***mycobacterial*** mutants having ***modified*** protein ***production*** capabilities. In one embodiment, the mutants overexpress ***heat*** ***shock*** protein. In a specific embodiment, the ***mycobacterial*** mutant overexpresses ***heat*** ***shock*** proteins 60 and/or 70. Also provided are modified BCG vaccines capable of overexpressing ***heat*** ***shock*** proteins 60 and/or 70.

AB . . . prevention of infectious diseases are provided. In particular, efficient vaccines comprising genetically modified pathogens are provided. The vaccines generally comprise ***mycobacterial*** mutants having ***modified*** protein ***production*** capabilities. In one embodiment, the mutants overexpress ***heat*** ***shock*** protein. In a specific embodiment, the ***mycobacterial*** mutant overexpresses ***heat*** ***shock*** proteins 60 and/or 70. Also provided are modified BCG vaccines capable of overexpressing ***heat*** ***shock*** proteins 60 and/or 70.

SUMM . . . the invention relates to the manipulation of antigen production by infectious organisms. More particularly, the present invention comprises manipulation of ***mycobacterial*** genes resulting in the modification of ***heat*** ***shock*** protein production.

SUMM [0003] ***Mycobacterial*** infections often manifest as diseases such as tuberculosis. Human infections caused by ***mycobacteria*** have been widespread since ancient times, and tuberculosis remains a leading cause of death today. Although the incidence of the disease declined in parallel with advancing standards of living since at least the mid-nineteenth century, ***mycobacterial*** diseases still constitute a leading cause of morbidity and mortality in countries with limited medical resources and can cause overwhelming, disseminated disease in immunocompromised patients. In spite of the efforts of numerous health organizations worldwide, the eradication of ***mycobacterial*** diseases has never been achieved, nor is

eradication imminent. Nearly one third of the world's population is infected with M. . . .

SUMM [0005] Approximately half of all patients with acquired immune deficiency syndrome (AIDS) will acquire a ***mycobacterial*** infection, with TB being an especially devastating complication. AIDS patients are at higher risks of developing clinical TB and anti-TB. .

SUMM [0006] ***Mycobacteria*** other than M. tuberculosis are increasingly found in opportunistic infections that plague the AIDS patient. Organisms from the M. avium-intracellulare complex (MAC), especially serotypes four and eight, account for 68% of the ***mycobacterial*** isolates from AIDS patients. Enormous numbers of MAC are found (up to 10.sup.10 acid-fast bacilli per gram of tissue) and, . . .

SUMM [0007] Crohn's disease is a chronic inflammatory bowel disease characterized by transmural inflammation and granuloma formation. ***Mycobacterium*** avium subspecies paratuberculosis (M. paratuberculosis) causes a similar disease in animals. Johnes's disease, affecting cattle, causes estimated losses of \$1.5. . .

SUMM [0008] Cattle also suffer from infection with ***Mycobacterium*** bovis which causes a disease similar to tuberculosis. Control of infection is a serious herd management concern. This infection can. .

SUMM . . . of new therapeutic agents that are effective as vaccines and as treatments for disease caused by drug resistant strains of ***mycobacteria*** .

SUMM [0011] Although over 37 species of ***mycobacteria*** have been identified, more than 95% of all human infections are caused by six species of ***mycobacteria*** : M tuberculosis, M. avium-intracellulare, M. kansasii, M. fortuitum, M. chelonae, and M. leprae. The most prevalent ***mycobacterial*** disease in humans is tuberculosis (TB) which is caused by ***mycobacterial*** species comprising M. tuberculosis, M. bovis, or M. africanum (Merck Manual 1992). Infection is typically initiated by the inhalation of. . .

SUMM [0012] There is still no clear understanding of the factors which contribute to the virulence of ***mycobacteria*** . Many investigators have implicated lipids of the cell wall and bacterial surface as contributors to colony morphology and virulence. Evidence suggests that C-mycosides, on the surface of certain ***mycobacterial*** cells, are important in facilitating survival of the organism within macrophages. Trehalose 6,6' dimycolate, a cord factor, has been implicated for other ***mycobacteria*** .

SUMM [0014] Diagnosis of ***mycobacterial*** infection is confirmed by the isolation and identification of the pathogen, although conventional diagnosis is based on sputum smears, chest X-ray examination (CXR), and clinical symptoms. Isolation of ***mycobacteria*** on a medium takes as long a time as four to eight weeks. Species identification takes a further two weeks. There are several other techniques for detecting ***mycobacteria*** such as the polymerase chain reaction (PCR), ***mycobacterium*** tuberculosis direct test, or amplified ***mycobacterium*** tuberculosis direct test (MTD), and detection assays that utilize radioactive labels.

SUMM . . . and many times, the results are inaccurate as false positives are sometimes seen in subjects who have been exposed to ***mycobacteria*** but are healthy. In addition, instances of mis-diagnosis are frequent since a positive result is not observed only in active TB patients, but also in BCG-vaccinated persons and those who

had been infected with ***mycobacteria*** but have not developed the disease. It is hard therefore, to distinguish active TB patients from the others, such as. . . by the tuberculin skin test. Additionally, the tuberculin test often produces a cross-reaction in those individuals who were infected with ***mycobacteria*** other than M tuberculosis (MOTT). Diagnosis using the skin tests currently available is frequently subject to error and inaccuracies.

SUMM . . . no longer consistently effective as a result of the problems with treatment compliance contributing to the development of drug resistant ***mycobacterial*** strains.

SUMM . . . infectious organism genes resulting in the modification of protein production are provided. Specifically, the present invention provides a teaching of ***mycobacterial*** genetic manipulation which results in an increase in ***heat*** ***shock*** protein production. The increase in ***heat*** ***shock*** protein production results in an enhanced immune response to the ***heat*** ***shock*** proteins and also other ***mycobacterial*** proteins in general.

SUMM [0021] ***Heat*** ***shock*** proteins (***hsp***) are widely distributed in nature and are among the most highly conserved molecules of the biosphere. ***Heat*** ***shock*** proteins perform important functions in the folding and unfolding or translocation of proteins, as well as in the assembly and disassembly of protein complexes. Because of these helper functions, ***heat*** ***shock*** proteins have been termed molecular chaperones. ***Heat*** ***shock*** protein synthesis is increased to protect prokaryotic or eukaryotic cells from various insults during periods of stress caused by infection, . . .

SUMM . . . inventors of the present invention provide for the first time a teaching of the use of pathogenic, and more specifically ***mycobacterial*** , ***heat*** ***shock*** proteins in novel vaccines and therapeutics. The findings of the inventors are both unobvious and unexpected since those skilled in the art have not considered the use of ***heat*** ***shock*** proteins in this capacity. For example, Zugel et al. state that "although ***hsp*** play an important role in several infectious and autoimmune diseases, evidence arguing against the direct involvement of ***heat*** ***shock*** proteins in protection or autoaggression has been gathered. At present, initiation of protective immunity against infectious antigens or autoimmune disorders by ***heat*** ***shock*** proteins alone appears unlikely." (Zugel et al. Clinical Microbiology Reviews 12(1) pp 19-39 (1999) (emphasis added)).

SUMM [0024] The vaccination methods described herein involve the manipulation of ***mycobacterial*** protein production. Such proteins include, but are not limited to, ***mycobacterial*** ***heat*** ***shock*** proteins such as ***heat*** ***shock*** protein

60

(***Hsp60***) (GroEL1, Rv3417c:GroEL2, Rv0440), ***Hsp10*** (GroES, Rv3418c), ***Hsp70*** (Rv0350), DnaJ (***Hsp40*** , Rv0352), GrpE (Rv0351) and ClpB (Rv0384c) and ***Hsp90*** . A particularly preferred embodiment of the invention comprises a mutant strain of M. tuberculosis that constitutively overexpresses ***Hsp70*** . Another preferred embodiment of the present invention comprises M. bovis BCG (hereafter "BCG") vaccines capable of ***heat*** ***shock*** protein overexpression. In another preferred embodiment, mutant strains of ***mycobacteria*** or BCG overexpress more than one ***heat*** ***shock*** protein; such

mutants include for example, strains that overexpress both ***Hsp70*** and ***Hsp60***. The present invention contemplates other combinations of ***heat*** ***shock*** protein overexpression. The present invention further contemplates overexpression of other ***mycobacterial*** proteins such as antigenic proteins found in the cell wall or secreted by the pathogen.

SUMM [0026] Another object of the present invention is to provide methods and compositions for the treatment and prevention of ***mycobacterial*** disease such as tuberculosis.

SUMM [0027] It is another object of the present invention to provide methods and compositions for the treatment and prevention of ***mycobacterial*** disease using compositions comprising genetically altered ***mycobacteria*** that are capable of overexpressing certain proteins.

SUMM . . . present invention is to provide methods and compositions for the treatment and prevention of tuberculosis using compositions comprising genetically altered ***mycobacteria*** that overexpress certain proteins, wherein the proteins comprise ***heat*** ***shock*** proteins, cell wall proteins or other antigenic proteins secreted by the pathogen.

SUMM . . . to provide methods and compositions for the treatment and prevention of tuberculosis wherein the proteins overexpressed by the genetically altered ***mycobacteria*** comprise ***Hsp60***, ***Hsp70*** and various combinations thereof.

SUMM [0030] Another object of the present invention is to provide compositions for vaccine formulations for the prevention of ***mycobacterial*** disease.

SUMM [0032] Yet another object of the present invention is to provide compositions for vaccine formulations for the prevention of ***mycobacterial*** disease caused by ***mycobacterial*** species comprising M. tuberculosis complex, M. avium-intracellulare, M. kansasii, M. fortuitum, M. chelonae, M. leprae, M. africanum, and M. microti.

SUMM [0033] Another object of the present invention is to provide methods for the manipulation of pathogenic organisms, namely ***mycobacterial*** genes, resulting in the modification of protein production.

SUMM [0034] It is yet another object of the present invention to provide methods and compositions for production of ***mycobacterial*** mutants characterized by a defective ***heat*** ***shock*** response.

SUMM [0035] Another object of the present invention is to provide methods and compositions for production of ***mycobacterial*** mutants wherein the ***hspR*** gene of M. tuberculosis has been modified resulting in the overexpression of ***Hsp70***.

SUMM [0036] Another object of the present invention is to provide methods and compositions for production of ***mycobacterial*** mutants wherein the ***hspR*** gene of BCG has been modified resulting in the overexpression of ***Hsp70***.

SUMM [0037] Another object of the present invention is to provide methods and compositions for production of ***mycobacterial*** mutants wherein the hrCA gene of M. tuberculosis has been modified resulting in the overexpression of ***Hsp60***.

SUMM [0038] It is another object of the present invention to provide methods and compositions for production of ***mycobacterial*** mutants wherein the hrCA gene of M. bovis has been modified resulting in the overexpression of ***Hsp60***.

SUMM [0039] Yet another object of the present invention is to provide methods

and compositions for production of ***mycobacterial*** mutants wherein both the ***hspR*** and hrcA genes of M. tuberculosis have been modified resulting in the overexpression of both ***Hsp70*** , ***Hsp60*** and co-regulated proteins.

SUMM [0040] Another object of the present invention is to provide methods and compositions for production of ***mycobacterial*** mutants wherein both the ***hspR*** and hrcA genes of BCG have been modified resulting in the overexpression of both ***Hsp70*** , ***Hsp60*** and co-regulated proteins

DRWD [0043] FIG. 1. Structure, regulation and mutagenesis of the ***hsp70*** (dnaK) operon.

DRWD [0044] a. The ***hsp70*** operon comprises four open reading frames, preceded by two copies of the HAIR (***HspR*** Associated Inverted Repeat) element (HAIR1, 5'-CTTGAGCGGGTGCACTCATC-3' (SEQ ID.NO: 1) and HAIR2, 5'-GTTGAGTGCATCAGGCTCAGC-3'; (SEQ ID NO: 2) identity to the consensus. . .

DRWD [0045] b. Gel shift analysis of interactions between histidine-tagged recombinant ***HspR*** and a double-stranded oligonucleotide corresponding to the HAIR element. * ***HspR*** -HAIR complex. **Temperature-sensitive super-shifted band.

DRWD . . . product corresponding to grpE and dnaJ. Lane 1, .lambda. HindIII ladder; lane 2, M. tuberculosis H37Rv; lane 3, M tuberculosis ***hspR*** mutant.

DRWD [0047] FIG. 2. Constitutive overexpression of ***hsp70*** proteins in the ***HspR*** mutant.

DRWD [0048] a. Mapping of transcriptional start points for the ***hsp70*** operon using mRNA extracted from wild type BCG (WT) and the .DELTA. ***hspR*** mutant with and without ***heat*** ***shock*** .

DRWD [0049] b. SDS-PAGE analysis of [.sup.35S]-methionine-labeled proteins from wild type BCG (WT) and the .DELTA. ***hspR*** mutant with and without ***heat*** ***shock*** .

DRWD [0050] FIG. 3. Growth and survival of the .DELTA. ***hspR*** mutant in stationary phase, heat stress conditions and macrophages.

DRWD [0051] The .DELTA. ***hspR*** mutant (.nu.) was compared to wild type M. tuberculosis (.omicron.) with respect to growth in laboratory culture.

DRWD [0058] FIG. 4. Characterization of the .DELTA. ***hspR*** mutant in a chronic infection model.

DRWD [0059] Mice were infected with wild type M. tuberculosis (.omicron.) and the corresponding .DELTA. ***hspR*** mutant (.nu.) and the bacterial load assessed in homogenised lung and spleen tissues. Bacterial load in the spleen (a) and. . .

DRWD [0061] Histological examination of representative sections from the lungs of mice 14 weeks after infection with the .DELTA. ***hspR*** mutant (a) and wild type M. tuberculosis (b). Magnification, .times.1000.

DRWD [0062] FIG. 6. Infection with the .DELTA. ***hspR*** mutant increases IFN-.gamma. production by splenocytes.

DRWD [0063] Mice were infected with BCG .DELTA. ***hspR*** (.nu.) and wildtype BCG (.omicron.) and the immune response in splenocytes was analysed by ELISPOT and flow cytometry.

DRWD [0064] a. IFN-.gamma. ELISPOT of ***Hsp70*** -stimulated cells.

DRWD [0065] b. Ratio of ***Hsp70*** -specific IFN-.gamma. to IL-4 producing cells.

DRWD . . . 8. Southern blot of KpnI digested gDNA probed with HRCA1/HRCA2. Lane 1, hindIII digest of .lambda. DNA; lane2, M. tuberculosis .DELTA. ***hspR*** ; lane 3, M. tuberculosis .DELTA. ***hspR*** .DELTA.hrcA

DRWD [0070] FIG. 9. SDS-PAGE showing overexpressed ClpB, ***Hsp70*** ,
 Hsp60 and ***Hsp10*** (GroES) in the ***hspR*** and
 hrcA deleted strain. Lane 1, wild type M. tuberculosis H37Rv; lane 2, M.
 tuberculosis .DELTA. ***hspR*** .DELTA.hrcA

DRWD [0071] FIG. 10. Gene expression profiles of M. tuberculosis during
 heat ***shock*** and of M. tuberculosis lacking the
 transcriptional repressor, ***HspR*** . Scatter plots show log
 Cy5/Cy3 signal ratios against log total signal intensity where log
 ratios are centralised such that mean. . . zero. A, Expression of M.
 tuberculosis genes at 45.degree. C. (Cy5) versus 37.degree. C. (Cy3). B,
 Expression in M. tuberculosis .DELTA. ***hspR*** (Cy5) versus
 wild-type M. tuberculosis H37Rv (Cy3) at 37.degree. C. C, Expression in
 M. tuberculosis .DELTA. ***hspR*** complemented with a functional
 copy of ***hspR*** on the integrating plasmid pSMT168 (Cy5) versus
 wild-type M. tuberculosis H37Rv (Cy3) at 37.degree. C.

DRWD [0072] FIG. 11. Functional distribution of genes upregulated during
 heat ***shock*** . Frequency of genes among functional
 groups
 (<http://genolist.pasteur.fr/TubercuList/>) across the genome (grey bars) .
 and among ***heat*** ***shock*** induced genes (black bars).

DRWD [0073] FIG. 12. ***Heat*** ***shock*** repressor binding sites
 within M. tuberculosis. A, ***HspR*** associated inverted repeat or
 HAIR sequences. B, HrcA binding sites or CIRCE (controlling inverted
 repeat of chaperone expression).

DRWD [0074] FIG. 13. Deletion of hrcA and ***hspR*** results in
 overexpression of ***Hsp70*** (DnaK), ***Hsp60*** (GroEL),
 Hsp10 (GroES) and a protein consistent in size with Acr2. A,
 Southern blot of KpnI digested genomic DNA demonstrating deletion of
 hrcA in M. tuberculosis .DELTA. ***hspR*** . Lane 1, HindIII digested
 .lambda. DNA; lane 2, M. tuberculosis .DELTA. ***hspR*** (3634 bp
 wild-type hrcA hybridising fragment); lane 3, M. tuberculosis .DELTA.
 hspR .DELTA.hrcA (6526 bp hrcA-deleted fragment). B, Protein
 extracts of 37.degree. C. cultured M. tuberculosis H37Rv (lane 1) and M.
 tuberculosis .DELTA. ***hspR*** .DELTA.hrcA (lane 2) separated by
 SDS-PAGE and stained with coomassie brilliant blue.

DRWD [0075] FIG. 14. Table 1. Upregulated genes in M. tuberculosis .DELTA.
 hspR compared to wild-type H37Rv. cDNAs from the mutant and
 wildtype strains were labelled with Cy5 and Cy3 respectively and
 competitively. . . .

DRWD [0076] FIG. 15. Table 2 Upregulated genes in M. tuberculosis .DELTA.
 hspR .DELTA.hrcA compared to wild-type H37Rv. cDNAs from the
 mutant and wild-type strain were labelled with Cy5 and Cy3 respectively
 and competitively. . . .

DETD [0079] ***Mycobacterial*** infections such as those causing
 tuberculosis, once thought to be declining in occurrence, have rebounded
 and again constitute a serious. . . threat. Areas where humans are
 crowded together or living in substandard housing are increasingly found
 to have persons infected with ***mycobacteria*** . Persons who are
 immunocompromised are at great risk of being infected with
 mycobacteria and dying from such infection. In addition, the
 emergence of drug-resistant strains of ***mycobacteria*** has added
 to the treatment problems of such infected persons.

DETD [0080] Many people who are infected with ***mycobacteria*** are poor
 or live in areas with inadequate health care facilities. As a result of
 various obstacles (economical, education levels. . . these and other
 individuals results in the prevalence of disease frequently compounded

by the emergence of drug resistant strains of ***mycobacteria*** . Effective vaccines that target various strains of ***mycobacteria*** are necessary to bring the increasing numbers of tuberculosis under control.

DETD [0081] The present invention provides methods and compositions comprising genetically modified pathogenic organisms such as ***mycobacteria*** for the prevention and treatment of infectious disease such as tuberculosis. More particularly, the present invention provides ***mycobacterial*** mutants capable of altered protein expression. As described herein, the protein that has altered expression may be overexpressed and may comprise any relevant ***mycobacterial*** protein, such as a cell wall protein or other antigenic protein secreted by the pathogen. Typically, the overexpressed protein is a ***heat*** ***shock*** protein such as ***Hsp60*** or ***Hsp70*** . In an alternative embodiment of the present invention, `multiple` mutants i.e. genetically modified ***mycobacteria*** capable of altered expression of more than one protein, are also provided. In a particular embodiment, `double` mutants capable of overexpressing ***Hsp60*** and ***Hsp70*** related proteins, are provided.

DETD [0082] In addition to the above-described embodiments, the present invention also provides improved BCG vaccines capable of overexpressing ***heat*** ***shock*** proteins. In a most preferred embodiment,

a vaccine comprising BCG capable of overexpressing both ***Hsp60*** and ***Hsp*** 70 and co-regulated proteins is provided.

DETD [0083] The methods and compositions of the present invention may be used for vaccinating and treating ***mycobacteria*** infection in humans as well as other animals. For example, the present invention may be particularly useful for the prevention. . .

DETD [0084] As used herein the term "tuberculosis" comprises disease states usually associated with infections caused by ***mycobacteria*** species comprising M. tuberculosis complex. ***Mycobacterial*** infections caused by ***mycobacteria*** other than M. tuberculosis (MOTT) are usually caused by ***mycobacterial*** species comprising M. avium-intracellulare, M. kansasii, M. fortuitum, M. chelonae, M. leprae, M. africanum, M. microti and M. paratuberculosis.

DETD [0085] Elevated expression of ***heat*** ***shock*** proteins can benefit a microbial pathogen struggling to penetrate host defenses during infection, but at the same time may provide. . . To determine which of these effects predominate, the present inventors constructed a mutant strain of M. tuberculosis that constitutively overexpresses ***Hsp70*** proteins. Surprisingly, although the mutant was fully virulent in the initial stage of infection, it was significantly impaired in its ability to persist during the subsequent chronic phase. As demonstrated herein, the present inventors discovered that induction of microbial ***heat*** ***shock*** genes provides a novel strategy to boost the immune response of individuals harboring latent tuberculosis infection.

DETD [0086] Cells exposed to elevated temperature or other stress stimuli respond by increased expression of ***heat*** ***shock*** proteins..sup.1 The ***heat*** ***shock*** response, and the proteins involved, have been highly conserved throughout evolution from Escherichia coli to man. The major ***heat*** ***shock*** proteins are molecular chaperones with an essential role in directing folding and assembly of polypeptides within the cell..sup.2 Enhanced expression of ***heat*** ***shock*** proteins in response to stress allows cells to tolerate potentially harmful consequences

associated with intracellular accumulation of denatured polypeptides.
DETD [0087] Synthesis of ***heat*** ***shock*** proteins is induced in microbial pathogens during infection.sup.3-5. While the increased level of these proteins is likely to enhance microbial. . . have discovered that it may also provide an important signal in alerting the host to the presence of the pathogen. ***Heat*** ***shock*** proteins interact with the immune system through a variety of mechanisms. They were initially identified as prominent antigens in a. . . as chaperones is associated with an ability to promote immune responses to other polypeptides.sup.8,9. Finally, although the functional role of ***heat*** ***shock*** proteins is primarily intracellular, several studies suggest that exogenous ***heat*** ***shock*** proteins trigger immunomodulatory signals as a result of recognition by cell surface receptors.sup.10-12.

DETD [0088] Current knowledge in this area provides that ***heat*** ***shock*** proteins are mainly associated with disease and that these

proteins are "virulence factors" that constitute the part of the ***mycobacterial*** organism that is fundamentally responsible for disease. Contrary to current knowledge however, the present inventors have examined the role of. . . more than wildtype and caused less pathology. Accordingly, another important aspect of the present invention is that overexpression of the ***mycobacterial*** ***heat*** ***shock*** protein not only increases the immune response to that particular protein, but it also enhances the immune response to other ***mycobacterial*** proteins.

DETD [0089] The present study was designed to explore the apparent paradox that increased expression of ***heat*** ***shock*** proteins has the potential to benefit both the pathogen and the host during infection. The inventors focused on M. tuberculosis,. . . within the toxic environment of phagocytic cells, with the outcome of infection crucially dependent on the host cell-mediated immune response. ***Heat*** ***shock*** proteins were amongst the first antigens identified from M. tuberculosis.sup.7, and are currently under investigation as vaccine candidates.sup.14. The present experimental strategy was firstly to investigate the genetic basis of ***heat*** ***shock*** regulation in M. tuberculosis, and then to construct a mutant strain with a defective ***heat*** ***shock*** response. As described herein, the inventors have created novel M. tuberculosis mutants characterized by constitutive overexpression of ***Hsp70***, and/or ***Hsp60***, and related proteins, and demonstrated that this ultimately results in a bias in favor of the host rather than the. . .

DETD [0090] Although ***mycobacterial*** ***heat*** ***shock*** proteins have been used extensively in immunological experiments, relatively little attention has been given to regulation of the ***mycobacterial*** ***heat*** ***shock*** response. As detailed in the Examples section, the present inventors have demonstrated that ***Hsp70*** expression in M. tuberculosis is regulated by a repressor system analogous to that in Streptomyces.sup.24. The ***HspR*** repressor controls expression of only a small number of genes in M. tuberculosis, comprising the ***hsp70*** operon and the gene encoding the ATPase ClpB.sup.23,28, which like ***Hsp70*** is preceded by an inverted repeat resembling the HAIR element.

DETD [0091] In contrast to the toxic effect of ***Hsp70*** overexpression in E. coli.sup.29, constitutive overexpression of the ***Hsp70*** proteins resulted in only a slightly reduced growth rate of M.

tuberculosis under in vitro culture conditions. This is consistent with the relatively modest effect of ***hspR*** deletion on the in vitro phenotype of Streptomyces mutants.sup.30 and is presumably due to the extra metabolic load of increased protein production. Increased thermotolerance of M. tuberculosis .DELTA. ***hspR*** is consistent with the proposed function of ***Hsp70*** proteins in response to stress. In contrast, overexpression of ***heat*** ***shock*** proteins in E. coli was not on its own sufficient to increase thermotolerance.sup.31.

DETD [0092] The phenotype of the .DELTA. ***hspR*** mutant during murine infection is of considerable interest. The availability of tools for ***mycobacterial*** mutagenesis has allowed identification of a

number

of genes involved in virulence of M. tuberculosis. Most of these mutations result in defects in macrophage survival and during the acute phase of infection.sup.32-34. Two loci resemble ***hspR*** in generating mutants with defects specifically affecting the chronic, or persistent, phase of infection. Mutation in a cyclopropane synthetase gene interferes with lipid biosynthesis causing a change in the surface structure of the ***mycobacteria*** and affecting survival in the chronic phase.sup.35. Deletion of the gene encoding the enzyme isocitrate lyase similarly reduces persistence.sup.36. A . . . in this case is that utilization of fatty acid derived substrates via the glyoxylate pathway makes an essential contribution to

mycobacterial metabolism in the chronic phase of infection.

DETD . . . to be bound by the following theory, two general mechanisms can be proposed to account for reduced survival of the .DELTA. ***hspR*** mutant. Firstly, the high level of the ***Hsp70*** proteins within the cell may block some developmental program involved in ***mycobacterial*** adaptation. If, for example, persistence involves formation of some spore-like `dormant` form of the organism.sup.37, it is possible that this. . . survival under conditions inimical to replication. Survival in activated macrophages indicates that, in contrast to the isocitrate lyase mutant.sup.6, the .DELTA. ***hspR*** mutant is able to undergo metabolic adaptation required for survival in an acidified intracellular compartment.

DETD [0094] In an alternative and preferred theory, the present inventors propose that the .DELTA. ***hspR*** phenotype is immune mediated. This is consistent with the fact that it is evident only after the onset of the acquired immune response. There are several mechanisms by which increased expression of ***Hsp70*** might enhance immune recognition of the .DELTA. ***hspR*** mutant. By increasing the antigen load per bacterium, ***Hsp70*** overexpression may either prime a stronger immune response or make cells infected by the mutant more attractive targets for effector. . . of the mechanisms, the present inventors have successfully demonstrated an enhanced immune response as a result of exposure to the .DELTA. ***hspR*** mutant. Specifically the inventors have surprisingly shown that infection of mice with BCG .DELTA. ***hspR*** induces an increased number of ***Hsp70*** -specific IFN- γ secreting splenocytes in comparison to wild type BCG. The enhanced immune response observed under these conditions, presents ***mycobacterial*** mutants capable of overexpressing ***heat*** ***shock*** proteins as excellent candidates for use

in

novel vaccines and treatments for tuberculosis..sup.1

DETD [0095] In addition to recognition of the ***Hsp70*** protein itself, the chaperone function of ***Hsp70*** presents further potential for

immune enhancement. Although enhanced secretory production of a single-chain antibody fragment by coproduction of molecular chaperones has been observed in *Bacillus subtilis*.sup.38 constitutive overexpression of ***heat*** ***shock*** proteins in ***mycobacteria*** resulting in enhanced immune response has been demonstrated for the first time by the present inventors. Secretion of proteins from viable ***mycobacteria*** is thought to facilitate their early immune recognition and is used as a criterion for selection of candidate antigens for inclusion in subunit vaccines.sup.39. The findings of the present inventors demonstrate that the effect of ***Hsp70*** overexpression on protein secretion in vivo enhances immune responses to other ***mycobacterial*** proteins. ***Hsp70*** released from ***mycobacterial*** cells promotes presentation of ***mycobacterial*** antigens or antigen fragments attached to its peptide-binding site. Consistent with both of the above scenarios, infection of mice with BCG .DELTA. ***hspR*** induced an increased number of CD8.sup.+ IFN-.gamma. secreting T cells in the spleen. The increase in ***Hsp70*** -specific IFN-.gamma. producing cells was not in itself sufficient to account for this difference; there must be some other additional enhancement of CD8.sup.+ IFN-.gamma. responses and the enhanced immune response is attributed to the chaperone function of ***Hsp70***.

DETD [0096] Accordingly, the enhanced immune response observed following exposure to ***mycobacterial*** mutants overexpressing ***heat*** ***shock*** proteins is not solely a result of the increase in the amount of ***heat*** ***shock*** proteins present themselves, it is also thought to be a result of the chaperone function of the ***heat*** ***shock*** protein. Therefore, functions of proteins such as ***Hsp70*** in promoting the secretion of other ***mycobacterial*** proteins, promoting the immune presentation of other ***mycobacterial*** antigens and acting directly on immune cells inducing accessory immune signals, are also important characteristics of any ***heat*** ***shock*** protein overexpressing strain.

DETD [0097] While further analysis of the ***hspR*** mutant provides an opportunity to assess these different aspects of ***heat*** ***shock*** protein immunogenicity, the present study demonstrates that, on balance, ***Hsp70*** overexpression favors the host over the pathogen during the chronic phase of tuberculosis infection. With an estimated one third of the global population currently infected with *M. tuberculosis*.sup.41, interventions targeted against persistent ***mycobacteria*** could have profound public health impact.

Induction

of ***mycobacterial*** ***heat*** ***shock*** protein expression by specific disruption of ***HspR*** regulation or by promotion of protein denaturation, for example may provide a novel strategy for reinforcement of host defenses during.

DETD [0098] Microarray analysis of an ***hspR*** deletion mutant of *M. tuberculosis* confirms and extends the above-described studies of ***Hsp70*** regulation. ***HspR*** is a DNA-binding protein related to the MerR family. It recognises either of two inverted repeat sequences (HAIR) in the promoter region of the ***hsp70*** operon, reducing the level of transcription in unstressed conditions. The ***HspR*** protein interacts tightly with ***Hsp70*** in vitro..sup.47,68 A system where this heterodimer forms the functional repressor unit with feedback achieved by titration of ***Hsp70*** away from the ***HspR*** complex in the presence of unfolded

polypeptides represents an attractive model for regulation..sup.10,63 We show that in the absence of ***HspR*** there is release of transcriptional repression and the genes of the ***Hsp70*** operon are upregulated. Surprisingly, there were also a further 46 genes with significantly elevated transcription. Of these, only three genes. . . with a HAIR-like sequence. Interestingly, the lead gene Rv0251c has also been shown to be under the control of the ***heat*** - ***shock*** responsive ECF sigma factor, .sigma.E, and is also prominent in response to treatment with SDS..sup.61 This dual control mechanism may account for the relatively modest elevation of Rv0251c transcription in the .DELTA. ***hspR*** mutant compared to that observed under ***heat*** ***shock*** conditions in the wild-type.

DETD [0099] Rv0251c encodes a 159 amino acid protein belonging to the small ***heat*** ***shock*** protein family, termed ***Hsp20***, or the .alpha.-crystallin family. Its predicted size is consistent with the approximately 20kD protein observed by SDS-PAGE to be upregulated in the .DELTA. ***hspR*** .DELTA.hrcA mutant (FIG. 12B). The small ***heat*** ***shock*** proteins, like the larger ***heat*** ***shock*** protein families, are found widely in bacterial and eukaryotic cells and appear to function as molecular chaperones at least in. . . family member was originally identified as a prominent antigen and is variously referred to as the 14kD antigen, 16kD antigen, ***Hsp16*** .3, .alpha.-crystallin (Acr), or ***HspX***. This gene is not induced by ***heat*** ***shock***, but is upregulated in stationary phase cultures and during the hypoxic response..sup.51,67,77,78 It is possible that the different .alpha.-crystallin homologues. . .

DETD [0100] Within the .DELTA. ***hspR*** -upregulated ORF set, the ***Hsp70*** and Acr2 operon genes were upregulated during ***heat***

shock along with bfrB, groES and Rv3654c. The bacterioferritin gene, bfrB, and Rv3654c, encoding an 8kD protein with unknown function, are not preceded by obvious ***HspR*** binding sites, but their coregulation with HAIR-associated genes in both ***heat*** ***shock*** and the mutant suggest an indirect link to ***HspR***

The majority of genes upregulated in the mutant were neither associated with HAIR sequences nor were they upregulated during ***heat*** ***shock***. We conclude that the induction of these genes is a consequence of the physiological changes associated with overexpression of the ***HspR*** -regulated proteins and may not be directly relevant to the normal ***heat*** ***shock*** response. An interesting example of this was the trend for upregulation of ribosomal protein expression, which was also mirrored in the .DELTA. ***hspR*** .DELTA.hrcA strain.

DETD [0101] A surprising omission from the .DELTA. ***hspR*** upregulated list was clpB, which encodes another probable molecular chaperone. We have previously shown the elevation of ClpB expression in. . . proteomic analysis.sup.68 which suggests that the clpB mRNA is of a sufficiently short half life to preclude detection of the .DELTA. ***hspR*** -associated transcriptional increase. The detection of substantially increased clpB mRNA in the wild-type after ***heat*** ***shock*** at 45.degree. C. is explained by upregulation of clpB transcription by the heat inducible sigma factor, .sigma.H, as well as release of ***HspR*** repression..sup.66

DETD [0102] Though not wishing to be bound by the following theory, it is thought that release of ***HspR*** repression significantly

influences ***heat*** ***shock*** protein production and may therefore have a corresponding effect on the host immune system. The findings of ***heat*** ***shock*** protein manipulation are not limited to ***mycobacterial*** organisms, and may also be extrapolated to other infectious agents that express ***heat*** ***shock*** protein.

DETD [0104] In order to create mutants having altered expression of more than one ***mycobacterial*** protein a similar strategy as discussed above was employed to replace the hrcA gene (Rv2374c) in the .DELTA. ***hspR*** strains with the kanamycin resistance gene from Tn903 (kan). The plasmid pSMT99 contains an E.coli origin of replication, the kan. . . to make pSMT163 (FIG. 7). 1 .mu.g of plasmid was irradiated with 100 mj/cm.sup.2 UV and electroporated into M. tuberculosis .DELTA. ***hspR*** or BCG .DELTA. ***hspR***. Transformants resulting from double crossover integration of the kan gene were selected on 7H11/OADC medium containing 15 .mu.g/ml kanamycin and. . . band of approximately 3600 bp and gene replacement strains gave a band of approximately 6500 bp (FIG. 8). Overexpression of ***Hsp60*** and ***Hsp70*** associated proteins was confirmed by SDS-PAGE and coomassie staining of protein extracts from bacteria grown at 37.degree. C. in Middlebrook. . .

DETD [0105] Unmarked .DELTA. ***hspR*** .DELTA.hrcA strains will be generated using suicide plasmids containing the mutated but unmarked target gene, hyg, sacB and LacZ. The plasmid will be introduced to the ***mycobacteria*** as described above and single cross-over integrants

selected as hygromycin resistant (hygR), LacZ+(blue) colonies on hygromycin/X-gal medium. A single clone. . .

DETD [0106] We were able to delete the proposed hrcA gene in the .DELTA. ***hspR*** mutant but the same approach has been unsuccessful with wild-type M. tuberculosis. This may reflect some technical problem, but it is also possible that overexpression of ***Hsp70*** proteins compensates in some way for a deleterious effect of hrcA deletion. Upregulation of the major ***HspR*** -regulated genes was preserved in the double mutant, alongside upregulation of the HrcA regulon, which included the ***Hsp60*** family genes, groES, groEL1 and groEL2. GroES is functionally related to GroEL and its gene is situated immediately upstream of groEL1. While the expression of groES was enhanced in the .DELTA. ***hspR*** mutant, its upregulation in the .DELTA. ***hspR*** .DELTA.hrcA strain was much greater. The M. tuberculosis HrcA protein has yet to be analysed for DNA-binding in vitro, but it. . . and groEL2 promoter regions. Thus, we can conclude that the HrcA repressor acts as the main transcriptional controller of the ***Hsp60*** /GroE family ***heat*** ***shock*** response, with some cross-talk between the ***Hsp60*** and ***Hsp70*** responses demonstrated by the induction of GroES expression in the ***hspR*** deleted strain. The mechanism for this cross-talk is unclear although a weak match for the ***HspR*** binding site, HAIR, is present at the beginning of the GroES ORF. Interaction of ***HspR*** with this inverted repeat could conceivably have a more subtle effect on transcription than that observed with HAIR sequences that. . .

DETD [0107] A good match for the CIRCE sequence is found upstream of another .DELTA. ***hspR*** .DELTA.hrcA upregulated gene, Rv0991c, which encodes a conserved hypothetical protein with unknown function. Expression of both Rv0991c and the adjacent downstream ORF, Rv0990c, was elevated during ***heat*** ***shock*** but Rv0990c was not

significantly upregulated in the mutant. Whether the two genes are transcribed as a bicistronic message or. . . are separately regulated and transcribed remains to be conclusively determined. Thus, it is clear that HrcA regulates not just the ***Hsp60*** ***heat*** ***shock*** response but also Rv0991c and probably Rv0990c. In light of the effect of the .DELTA. ***hspR*** mutation on the virulence of M. tuberculosis.sup.68, it will be of considerable interest to study the double mutant in infection. . .

DETD . . . Based on these studies and the 45.degree. C. transcriptional snapshot, one skilled in the art may conclude that that the ***HspR*** and HrcA regulons, which dominate the ***heat*** ***shock*** proteome comprise only a part of the overall adaptive response. Genes regulated by .sigma.H and .sigma.E are prominent in the. . . and upregulation of the .sigma.B gene suggests overlap with the general stress response. These different regulatory layers are interlinked, with ***hsp70*** and clpB under dual ***HspR*** and .sigma.H control, and acr2 under dual ***HspR*** and .sigma.E control. Moreover, the heat inducible expression of .sigma.B and .sigma.E is dependent on .sigma.H which autoregulates its own. . .

DETD . . . above may be employed to create mutants continuing multiple modifications resulting in the overexpression of more than one or two ***heat*** ***shock*** proteins.

DETD [0111] Therapeutics including vaccines comprising ***mycobacterial*** mutants of the present invention, such as BCG overexpressing ***Hsp60*** and/or ***Hsp70***, can be prepared in physiologically

acceptable formulations, such as in pharmaceutically acceptable carriers, using known techniques. For example, the mutant. . .

DETD . . . may be administered in combination with other compositions and procedures for the treatment of other disorders occurring in combination with ***mycobacterial*** disease. For example, tuberculosis frequently occurs as a secondary complication associated with acquired immunodeficiency syndrome (AIDS). Patients undergoing treatment AIDS. .

DETD Characterisation of ***HspR***

DETD [0118] The ***hspR*** gene from M. tuberculosis was amplified by PCR from pY3111.sup.42 and ligated into pQE30 (Qiagen, West Sussex, U.K).. Transformants in. . . M urea-TBS, bound protein was renatured using a gradient from 6 M urea in TBS to TBS alone, and histidine-tagged ***HspR*** eluted with 250 mM imidazole in TBS.

DETD [0119] Binding of purified ***HspR*** to HAIR2 was tested in a gel shift assay using an .alpha.[sup.32P]-labelled double stranded oligonucleotide generated by annealing DNAKIR-F (5'- . . . PMSF, 20 .mu.g BSA, 2 .mu.g sonicated salmon sperm DNA, 20% glycerol, 300 pg labelled oligonucleotide and 150 ng His-tagged ***HspR*** with or without 10 .mu.g BCG sonicated cell extract. Products were electrophoresed in 6% native polyacrylamide and migration visualised by.

DETD Generation and Characterization of .DELTA. ***hspR*** Mutants

DETD [0120] DNA fragments (2 kb) immediately upstream and downstream of ***hspR*** were amplified with Pwo polymerase using the primer pairs HS1(5'-GGACTAGTCGTTGTGGACGCGAGGTG-3') (SEQ ID NO: 10) /HS2(5'-GCTCTAGACCCCGTCCTTTGGGTTCTTC-3') (SEQ ID NO: 11). . .

DETD . . . gene, and gene replacement transformants were selected as described previously.sup.43. In attempts to restore the wild type phenotype, the cloned ***hspR*** gene was reintroduced into M. tuberculosis on plasmid vectors under the control of the constitutively

active superoxide dismutase (sodA) promoter, . . .

DETD [0123] Transcriptional start sites were located using RNA extracted from cultures of BCG and the corresponding .DELTA. ***hspR*** mutant grown at 37.degree. C., with or without ***heat*** ***shock*** for 45 min at 45.degree. C., as described by Mangan et al..sup.15. .gamma.[.sup.32P]-labelled primer (PEXI, 5'-CCTCCTGAATATGTAGAG-3') (SEQ ID NO: 14).

DETD [0126] Bone marrow-derived macrophages were cultivated and infected with ***mycobacteria*** as previously described.sup.43 but using Macrophage-SFM Medium (Life Technologies) supplemented with 10 ng/ml IL-3 (Pharmingen, Franklin Lakes, N.J., U.S.A.).

DETD [0128] C57BL/6 mice were infected intravenously with 2.times.10.sup.5. CFU BCG or BCG .DELTA. ***hspR***. Animals were culled at day 14 and 35 after infection by intraperitoneal injection of 3 mg pentobarbitone and exanguination via. . . .

DETD . . . cells/well with 4 doubling dilutions. Cells were cultured for 48 hours with medium alone or 10 .mu.g/ml purified M. tuberculosis ***Hsp70*** .sup.42. The sites of cytokine production were detected using biotin-labelled rat antimurine IL-4, or IFN-.gamma. monoclonal antibodies (Pharmingen) as previously described.sup.45.

DETD Characterisation of ***Hsp70*** Regulation in M. Tuberculosis

DETD [0132] Exposure of M. tuberculosis to increased temperature results in elevated transcription of ***heat*** ***shock*** genes and expression of the corresponding proteins.sup.15,16. The regulatory mechanisms involved have not been characterized. Two general mechanisms for ***heat*** ***shock*** regulation have been identified in bacteria. Induction of the response in E. coli involves transcriptional activation, with increased levels of an alternative sigma factor, sigma-32, directing RNA polymerase towards genes preceded by a consensus ***heat*** ***shock*** promoter sequence.sup.17. In contrast, in Bacillus subtilis the ***heat*** ***shock*** response is regulated by transcriptional repression.sup.18. In unstressed cells, the HrcA repressor blocks transcription by binding to an inverted repeat element: upstream of the ***heat*** ***shock*** genes, with repression being released in response to stress stimuli. Inspection of the genome sequence of M. tuberculosis.sup.19 suggests repression as the probable mechanism of ***heat*** ***shock*** regulation. Open reading frame Rv2374c encodes a homologue of the HrcA repressor, while Rv0353 encodes a protein similar to ***HspR***, a repressor identified in ***Hsp70*** regulation in Streptomyces.sup.20 and in Helicobacter pylori.sup.21. The M. tuberculosis ***hspR*** is the fourth gene in an operon comprising ***Hsp70***, followed by genes encoding GrpE and DnaJ, ***heat*** ***shock*** proteins that have functional interactions with ***Hsp70*** .sup.22 (FIG. 1a).

DETD [0133] To test whether M. tuberculosis ***HspR*** has a function analogous to the Streptomyces homologue, it was expressed as a His-tagged protein and characterized in a gel shift assay (FIG. 1b). ***HspR*** bound to a 40 bp oligonucleotide corresponding to a region upstream of M. tuberculosis ***Hsp70*** containing a partial match for the ***HspR*** -associated inverted repeat (HAIR) identified in Streptomyces.sup.20,23. ***HspR*** showed no binding to a control irrelevant oligonucleotide. The effect of ***heat*** ***shock*** on the ***HspR*** -HAIR interaction was tested by carrying out the reaction at 48.degree. C. Heating had no effect on the gel shift pattern. An effect of ***heat*** ***shock*** was observed, however, when a ***mycobacterial*** extract was included in the assay. Reaction of the oligonucleotide with ***HspR*** and the cell

extract at low temperature, 30.degree. C., produced a second gel shift band (FIG. 1b, lane 3). This. . .

DETD [0134] The ability to bind to the upstream regulatory sequence suggests that *M. tuberculosis* ***HspR*** has a function analogous to that of its *Streptomyces* counterpart.^{sup.20} The presence of the temperature-sensitive super-shifted band is consistent with a model in which ***HspR*** and ***Hsp70*** together form the functional repressor, with sequestration of ***Hsp70*** as a result of binding to denatured proteins releasing repression during ***heat*** ***shock***.^{sup.24}

DETD Deletion of the ***HspR*** Repressor

DETD [0135] Taking advantage of *sacB* counter-selection.^{sup.25}, an allele replacement strategy was used to substitute the ***hspR*** gene with a hygromycin resistance cassette in *M. tuberculosis* and BCG (FIG. 1c).

DETD [0136] Expression of the ***hsp70*** operon in wild type *M. bovis* BCG and the .DELTA. ***hspR*** mutant was compared by mapping of transcriptional start points (FIG. 2a) In the wild type strain grown at 37.degree. C.,. . . in cells that had been heat shocked. In the mutant, transcription occurred from both sites even in the absence of ***heat*** ***shock***. TSP1 and TSP2 are located 5 bases and 6 bases upstream of HAIR1 and HAIR2 respectively. While transcription from both sites is therefore likely to be influenced by ***HspR***, the mapping results demonstrate that this effect is more pronounced in the case of the TSP2 transcript.

DETD [0137] Next the pattern of protein expression in the .DELTA. ***hspR*** mutants was analyzed. The response was the same in *M. tuberculosis* and BCG. The SDS-PAGE profiles of newly synthesised proteins labeled with [^{sup.35S}]-methionine at 37.degree. C. and 45.degree. C. (FIG. 2b) showed that ***Hsp70*** was induced in the wild type strains at the elevated temperature. In the mutants, however, this band was equally prominent. . . 37.degree. C. cultures. Other less marked differences included constitutive overexpression of bands at 90 kDa and 45 kDa in the .DELTA. ***hspR*** mutants, again corresponding to changes induced by ***heat*** ***shock*** in the wild type. The changes in protein profile were further characterized by two-dimensional gel electrophoresis. Three protein spots were upregulated in the mutant and were identified by peptide mass fingerprinting as ***Hsp70***, ClpB, and GrpE. DnaJ, the third ***heat*** ***shock*** protein in the ***hsp70*** operon, has a relatively basic isoelectric point (predicted pI 8.05) and was not resolved.

DETD [0138] Results generated using the deletion mutants were again consistent with the model in which ***HspR*** acts as a repressor of the ***hsp70*** operon. To confirm that the effects were due solely to the loss of ***hspR***, the cloned gene was reintroduced using ***mycobacterial*** expression vectors. These experiments were unsuccessful. Plasmids constitutively expressing ***HspR*** could not be maintained in ***mycobacteria***. Although it was possible to introduce the ***hspR*** gene into *M. tuberculosis* using the inducible acetamidase promoter.^{sup.6}, induction of ***HspR*** expression resulted in cessation of bacterial growth. Thus, while deletion of ***hspR*** is well-tolerated by *M. tuberculosis*, it seems that inappropriately regulated expression has a profound detrimental effect on bacterial viability. The location of the ***hspR*** gene at the end of the ***hsp70*** operon, and its reverse orientation with respect to the adjacent downstream PPE gene (Rv0354c) (FIG. 1a), suggests that polar effects. . .

DETD [0139] Phenotype of the .DELTA. ***hspR*** mutant in vitro and during

infection The M. tuberculosis and BCG mutants were slightly impaired for in vitro growth. Colonies. . . cultures was indistinguishable from wild type controls (FIG. 3a). A significant difference was observed in thermotolerance, with survival of the .DELTA. ***hspR*** mutant at 53.degree. C. enhanced in comparison to that of the parent strain (FIG. 3b).

DETD [0140] The M. tuberculosis .DELTA. ***hspR*** mutant was compared to the parent strain in its ability to survive in murine bone marrow macrophages. Both mutant and. . .

DETD [0141] Next, the ability of the .DELTA. ***hspR*** mutant to cause progressive infection in C57BL/6 mice was examined. In this model, the bacteria were seeded in multiple organs. . . lungs of the same animals, with a 1-2 log reduction in bacterial load in the mice infected with M. tuberculosis .DELTA. ***hspR*** at 14-weeks (P=0.016) (FIG. 4b). For accurate assessment of the low numbers of bacteria during the initial phase of infection in. . .

DETD . . . associated with increased immune-mediated pathology. The mean weight of animals at 10 and 14 weeks was slightly higher in the .DELTA. ***hspR*** group (25.35 g) compared to wild type (23.92 g) (P=0.058). Histological examination of lungs from .DELTA. ***hspR*** mice revealed small, isolated macroscopic lesions consisting mainly of macrophages with scattered lymphocytes and polymorphonuclear leucocytes (FIG. 5a). The majority. . .

DETD Immune Response to the .DELTA. ***hspR*** Mutant

DETD [0143] To test the hypothesis that reduced survival of M. tuberculosis .DELTA. ***hspR*** during chronic infection could be due to a heightened immune response, the effect of ***Hsp70*** overexpression on immunogenicity was investigated. Immune responses of mice infected intravenously with wild-type or BCG .DELTA. ***hspR*** were analyzed. As with M. tuberculosis, the wild-type and mutant strains survived similarly during acute infection, with no significant difference in CFUs at day 14. ELISPOT analysis of ***Hsp70*** -stimulated splenocytes at day 35 revealed a two-fold increase in the number of IFN-.gamma. producing cells from mice infected with BCG .DELTA. ***hspR*** compared to wild type (P=0.02) (FIG. 6a). The ratio of IFN-.gamma.:IL-4 producing ***Hsp70*** -specific splenocytes was also increased two-fold following BCG .DELTA. ***hspR*** infection (P=0.02) (FIG. 6b). Analysis of cell populations by flow cytometry did not reveal any significant difference in the number. . . in the spleen the number of CD8.sup.+ (but not CD4.sup.+) T cells secreting IFN-.gamma. was significantly higher in the BCG .DELTA. ***hspR*** infected group (P=0.009) (FIG. 6c). This increase in CD8.sup.+ IFN-.gamma. producing cells was larger than could be explained solely by the increase in ***Hsp70*** -specific IFN-.gamma. secreting cells observed by ELISPOT.

DETD Dissection of the ***Heat*** ***Shock*** Response to M. tuberculosis Using Mutants and Microarrays

DETD . . . C. in Luria Bertani broth and agar containing 150 .mu.g/ml hygromycin or 50 .mu.g/ml kanamycin where appropriate. M. tuberculosis H37Rv, .DELTA. ***hspR*** and .DELTA. ***hspR*** .DELTA.hrcA were grown at 37.degree. C. in Middlebrook 7H9 broth (Difco) containing 10% albumin dextrose catalase (ADC) enrichment or on. . . .mu.g/ml and kanamycin at 15 .mu.g/ml were added where appropriate. 2% sucrose was added to media for counterselection of sacB. ***Heat***

shock was performed by splitting 20 ml broth cultures at late log phase into two universal tubes and placing one tube. . .

DETD [0147] Deletion of ***hspR***, hrcA in M. tuberculosis

DETD [0148] The gene replacement of ***hspR*** with the hygromycin B

phosphotransferase gene (hyg) from *Streptomyces hygroscopicus* has been previously described..sup.68 The sequential deletion of *hrcA* to generate a double ***hspR*** *hrcA* mutant strain was achieved using a similar suicide delivery strategy but replacing the target gene, *hrcA*, with the kanamycin. . . Tn903. Briefly, 1.5 kb regions of DNA up and downstream of *hrcA* were cloned around the *aph* gene in the

mycobacterial suicide plasmid pSMT99 to make pSMT163. This plasmid cannot replicate in ***mycobacteria*** and carries *sacB* for counterselection against single crossover and illegitimate integration of the plasmid. 1 .mu.g of plasmid was irradiated with 100 mj/cm.sup.2 UV58 and electroporated into *M. tuberculosis* or *M. tuberculosis* .DELTA.

hspR ..sup.72 Following overnight recovery of the cells in 7H9/ADC, gene replacement transformants were directly selected on 7H11/OADC containing hygromycin, kanamycin and. . .

DETD [0149] Complementation of *M. tuberculosis* .DELTA. ***hspR***

DETD . . . based E.coli plasmid which carries the *aph* kanamycin resistance gene and the *int* gene and *attP* site from the L5

mycobacteriophage ..sup.69 This plasmid integrates into the chromosome in single copy by site-specific recombination at the *attB* site. The ***Hsp70*** operon promoter containing the two HAIR-regulated promoter regions.sup.68 was amplified by PCR using the primers ***Hsp701*** (tcggtcaagctggcggactga) (SEQ ID NO: 14) and ***Hsp702*** (agccatggtgaatcctcctg) (SEQ ID NO: 15) and cloned into the *SacI* site of pKinta. The ***hspR*** ORF was then amplified and cloned downstream of the ***hsp70*** promoter so as to transcriptionally fuse the ORF with its own promoter albeit without the intervening ***hsp70***, *grpE* and *dnaJ* sequence. The resultant plasmid, pSMT168, was introduced to *M. tuberculosis* .DELTA. ***hspR*** by electroporation.

DETD . . . calculated for each ORF in the mutant:wild-type comparisons through an ANOVA analysis. Each of the three data sets (wild-type v .DELTA. ***hspR***; wild-type v .DELTA. ***hspR*** pSMT168; wild-type v .DELTA. ***hspRhrcA***) forms a balanced factorial design. Three main effects were taken into account: the array effect A for each array, the. . .

DETD [0162] Overview of the *M. tuberculosis* ***Heat*** ***Shock*** Response

DETD [0163] Previous reports have described the induction of ***heat*** ***shock*** proteins in cultures of *M. tuberculosis* exposed to temperatures ranging from 37-48.degree. C. for varying lengths of time, and demonstrated transcriptional regulation of selected ***heat*** ***shock*** genes..sup.65,76 These studies demonstrate a complex response, which varies with both temperature and time of exposure. To obtain an overview of the ***heat*** ***shock*** response, we used whole genome microarray analysis to generate a transcriptomic snap-shot of the changes induced by incubation at 45.degree. C. for 30 minutes; conditions previously demonstrated to result in high level expression particularly of the ***Hsp70*** regulon. This is displayed in the scatter plot (FIG. 10A), which shows the global nature of the transcriptional changes induced by ***heat*** ***shock***; the expression ratio of many genes lying away from the zero line demonstrating altered expression. A list of the 100. . . genes, and away from cell wall associated genes (FIG. 11). The induced genes included all the known members of the ***HspR*** regulon, as well as the *groEL* and *groES* genes and other previously identified ***heat*** ***shock*** inducible genes including those encoding the alternative sigma factors .sigma.B, .sigma.H and .sigma.E..sup.52,60 This set of

heat-inducible genes included five. . . consensus promoter regions..sup.66 This is consistent with identification of these sigma factors as both heat-inducible genes and regulators of the ***heat***
shock response. To characterize regulation of genes encoding

the

major ***heat*** ***shock*** proteins, we next extended the microarray approach to analysis of mutant strains of M. tuberculosis from which predicted transcriptional repressors. . .

DETD

[0164] The ***HspR*** Regulon

DETD

[0165] By examining the gene expression profile at 37.degree. C. of an M. tuberculosis strain lacking the transcriptional repressor

HspR (.DELTA. ***hspR***), we aimed to isolate any de-repressed genes and identify the subset of heat inducible genes directly under ***HspR*** control. In contrast to the heat shocked bacteria, transcription of the majority of genes was unaltered in the mutant strain,. . . obvious upregulated genes, exposing a set of 49 upregulated ORFs (p<0.01) in the mutant strain, including the members of the ***Hsp70*** operon (dnaK, grpE and dnaJ) (FIG. 14, Table 1).

DETD

[0166] We searched the genome for sequences that resembled the ***HspR*** binding site, HAIR (***HspR*** Associated Inverted Repeat) CTTGAGT-N7-ACTCAAG (SEQ ID NO: 3).sup.53, and compared the locations of potential sites to the gene expression analysis of both heat shocked M. tuberculosis and M. tuberculosis .DELTA. ***hspR*** . In addition to the HAIR sequences already identified upstream of the ***Hsp70*** operon and clpB.sup.68, a HAIR-like domain was present 71 bp upstream of the start codon of Rv0251c (FIG. 12A). This. . . to encode an oxidoreductase) being members of the operon. Neither of these genes was detected as significantly upregulated in the .DELTA.

hspR mutant by ANOVA analysis. There were no other HAIR-like sequences associated with any of the other up-regulated genes in the .DELTA. ***hspR*** strain.

DETD

[0167] As expected the ***Hsp70*** operon genes along with acr2 and Rv0250c were upregulated in response to ***heat*** ***shock*** . Under the conditions used in this study, acr2 was the most heat inducible gene in the genome (FIG. 10A). Other .DELTA. ***hspR*** -regulated ORFs demonstrated to be induced under ***heat***

shock were Rv3654c, bfrB and groES. Rv3654c encodes an 8 kD protein of unknown function and bfrB encodes a bacterioferritin involved. . . an identifiable HAIR like sequence in its vicinity and both are therefore concluded to be under some indirect control by

HspR . Most interesting, is the inclusion of the chaperone gene groES as our previous studies had not indicated that this gene was controlled by ***HspR*** . Indeed the level of induction is considerably less than that of the ***Hsp70*** or Acr2 operons. The ***HspR*** associated control over groES expression may be indirect

as

there is no HAIR sequence in the promoter region, however there. . . a weak HAIR-like sequence situated 24 bases downstream of the groES initiation codon. The remaining non-heat-induced genes upregulated in the .DELTA. ***hspR*** mutant presumably reflect adaptive responses triggered by constitutive overexpression of the genes normally controlled by ***HspR*** . Notable members of this group included genes encoding the alternative sigma factor .sigma.C, the sec-independent protein translocase, TatA, and also. . . ribosomal proteins. Indeed, there was a general trend among nearly all the ribosomal protein genes to be upregulated in the .DELTA. ***hspR*** mutant.

DETD [0168] We had previously described unsuccessful attempts to complement the *M. tuberculosis* .DELTA. ***hspR*** strain..sup.68 Reintroduction of the gene with a constitutive promoter or even gently induced expression from the acetamidase promoter.sup.64 rendered the bacteria non-viable. These findings suggest that expression of reintroduced ***hspR*** would have to be appropriately regulated so as to closely match wild-type expression dynamics. To achieve this, the ***hspR*** gene was cloned under the control of the natural promoter of the ***hsp70*** operon, which includes two HAIR sequences. A single copy of this construct was inserted at the attB phage integration site in the chromosome of *M. tuberculosis* .DELTA. ***hspR***. In contrast to previous attempts at complementation, this strain was fully viable. Whole-genome expression profiling of the complemented mutant showed a pattern largely similar to the original wild-type strain (FIG. 10C). The reintroduced ***hspR*** gene was approximately 2-fold over-expressed demonstrating that the complementing construct did not express ***hspR*** identically to wild-type, perhaps reflecting some stoichiometric relationship between ***hspR*** expression and the number of HAIR sites. However, all the genes overexpressed in the .DELTA. ***hspR*** strain showed a complete or substantial reduction of overexpression in the complemented strain (FIG. 14, Table 1). This demonstrates that the altered transcriptome of the mutant was specifically due to the absence of ***hspR*** and not to polar effects on neighboring genes or to an inadvertently selected mutation.

DETD [0170] ORF Rv2374c in the *M. tuberculosis* genome shares sequence homology with the family of ***heat*** ***shock*** repressors related to the *hrca* gene of *B. subtilis*. To test whether this ORF is similarly involved in ***heat*** ***shock*** regulation in *M. tuberculosis* we undertook a deletion strategy analogous to that used to generate the .DELTA. ***hspR*** mutant, replacing *hrca* with a kanamycin resistance gene. We were unable to generate .DELTA.*hrca* mutants in wild-type *M. tuberculosis*, yet were successful at introducing the mutation into *M. tuberculosis* .DELTA. ***hspR*** (FIG. 13A). SDS-PAGE analysis of the total protein profile of the double knock out *M. tuberculosis* .DELTA. ***hspR*** .DELTA.*hrca* demonstrated constitutive overexpression of proteins consistent in size with ***Hsp70***, ***Hsp60*** (GroEL) and GroES, as well as an additional band at approximately 20 kD (FIG. 13B).

DETD [0171] Whole-genome expression profiling of *M. tuberculosis* .DELTA. ***hspR*** .DELTA.*hrca* at 37.degree. C. revealed enhanced expression

of

a set of 48 ORFs ($p < 0.01$) (FIG. 15, Table 2). Twelve ORFs upregulated in the single .DELTA. ***hspR*** mutant were also upregulated in the .DELTA. ***hspR*** .DELTA.*hrca* strain. These included members of the ***Hsp70*** and *Acr2* operons as well as *sigC*, *tatA* and *groES*. The upregulation of *groES* was much greater in the .DELTA. ***hspR*** .DELTA.*hrca* mutant than in the .DELTA. ***hspR*** strain (9.60 and 1.96 fold respectively). This indicated that although transcription of *groES* can be induced by an ***HspR***-associated mechanism, the predominant mode of transcriptional control is through the *HrcA* repressor. *HrcA* also seemed the likely mechanism of control for the two *M. tuberculosis* *groEL* genes as these were both strongly upregulated in the .DELTA. ***hspR*** .DELTA.*hrca* strain. We searched the genome for the *HrcA* binding site, CIRCE TTAGCACTC-N9-GAGTGCTAA (SEQ ID NO: 16).sup.56 and, as for ***HspR***, compared the putative CIRCE locations with both the ***heat*** ***shock*** expression data and the double mutant transcriptional profile. *groEL2* is preceded by two

CIRCE-like elements and groES/groEL1 by one (FIG. 12B). This confirmed the hypothesis that HrcA acts as the main regulator for the GroE/

- ***Hsp60*** ***heat*** ***shock*** protein family.
- DETD . . . (FIG. 12B). This ORF is predicted to encode an 11.5 kD conserved hypothetical protein and was significantly upregulated in the .DELTA. ***hspR*** .DELTA.hrcA mutant (FIG. 15, Table 2). Both Rv0991c and the immediately adjacent downstream gene Rv0990c were upregulated after ***heat*** ***shock*** for 30 min at 45.degree. C. in the wild-type. Although no significant change was detected in transcription of Rv0990c in the mutant strain, this suggests that the two genes may be coregulated. None of the remaining .DELTA. ***hspR*** .DELTA.hrcA upregulated genes were associated with CIRCE-like elements nor were they induced under ***heat*** ***shock*** in the wild-type. Similarly to the single .DELTA. ***hspR*** mutant there was a trend for ORFs encoding ribosomal proteins to be upregulated, but in addition the gene encoding ribosome. . . .
- DETD [0174] 1. Lindquist, S. & Craig, E. A. The ***heat*** - ***shock*** proteins. Annu Rev Genet 22, 631-677 (1988).
- DETD [0177] 4. Lee, B. Y. & Horwitz, M. A. Identification of macrophage and stress-induced proteins of ***Mycobacterium*** tuberculosis. J. Clin Invest 96, 245-249 (1995).
- DETD [0181] 8. Cho, B. K. et al. A proposed mechanism for the induction of cytotoxic T lymphocyte production by ***heat*** ***shock*** fusion proteins. Immunity 12, 263-272 (2000).
- DETD [0182] 9. Suto, R. & Srivastava, P. K. A mechanism for the specific immunogenicity of ***heat*** ***shock*** protein-chaperoned peptides. Science 269, 1585-1588 (1995).
- DETD [0183] 10. Arnold-Schild, D. et al. Cutting edge: receptor-mediated endocytosis of ***heat*** ***shock*** proteins by professional antigen-presenting cells. J. Immunol 162, 3757-3760 (1999).
- DETD [0184] 11. Asea, A. et al. ***HSP70*** stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. Nat Med 6, 435-442. . . .
- DETD [0185] 12. Castellino, F. et al. Receptor-mediated uptake of Antigen/ ***Heat*** ***shock*** protein complexes results in major histocompatibility complex class I antigen presentation via two distinct processing pathways. J Exp Med 191,
- DETD [0186] 13. Srivastava, P. K., Menoret, A., Basu, S., Binder, R. J. & McQuade, K. L. ***Heat*** ***shock*** proteins come of age: primitive functions acquire new roles in an adaptive world. Immunity 8, 657-665 (1998).
- DETD . . . M., Mitchison, D. A. & Butcher, P. D. An effective method of RNA extraction from bacteria refractory to disruption, including ***mycobacteria*** . Nucleic Acids Res 25, 675-676 (1997).
- DETD [0189] 16. Young, D. B. & Garbe, T. R. ***Heat*** ***shock*** proteins and antigens of ***Mycobacterium*** tuberculosis. Infect Immun 59, 3086-3093 (1991).
- DETD . . . D., Erickson, J. W. & Gross, C. A. The htpR gene product of E. coli is a sigma factor for ***heat*** - ***shock*** promoters. Cell 38, 383-390 (1984).
- DETD [0191] 18. Hecker, M., Schumann, W. & Volker, U. ***Heat*** - ***shock*** and general stress response in Bacillus subtilis. Mol Microbiol 19, 417-428 (1996).
- DETD [0192] 19. Cole, S. T. et al. Deciphering the biology of ***Mycobacterium*** tuberculosis from the complete genome sequence. Nature 393, 537-544 (1998).
- DETD . . . Bucca, G., Hindle, Z. & Smith, C. P. Regulation of the dnaK

operon of *Streptomyces coelicolor* A3(2) is governed by ***HspR*** , an autoregulatory repressor protein. J. Bacteriol 179, 5999-6004 (1997).

DETD [0194] 21. Spohn, G. & Scarlato, V. The autoregulatory ***HspR*** repressor protein governs chaperone gene transcription in *Helicobacter pylori*. Mol Microbiol 34, 663-674 (1999).

DETD [0195] 22. Liberek, K., Marszalek, J., Ang, D., Georgopoulos, C. & Zylicz, M. *Escherichia coli* DnaJ and GrpE ***heat*** ***shock*** proteins jointly stimulate ATPase activity of DnaK. Proc Natl Acad Sci U.S.A. 88, 2874-2878 (1991).

DETD [0196] 23. Grandvalet, C., de Crecy-Lagard, V. & Mazodier, P. The ClpB ATPase of *Streptomyces albus* G belongs to the ***HspR*** ***heat*** ***shock*** regulon. Mol Microbiol 31, 521-532 (1999).

DETD [0197] 24. Bucca, G., Brassington, A. M., Schonfeld, H. J. & Smith, C. P. The ***HspR*** regulon of *streptomyces coelicolor*: a role for the DnaK chaperone as a transcriptional co-repressordagger. Mol Microbiol 38, 1093-1103. (2000).

DETD . . . 25. Pelicic, V., Reyrat, J. M. & Gicquel, B. Expression of the *Bacillus subtilis* sacB gene confers sucrose sensitivity on ***mycobacteria*** . J. Bacteriol 178, 1197-1199 (1996).

DETD . . . Parish, T., Mahenthiralingam, E., Draper, P., Davis, E. O. & Colston, M. J. Regulation of the inducible acetamidase gene of ***Mycobacterium*** smegmatis. Microbiology 143, 2267-2276 (1997).

DETD . . . E. R., Frank, A. A. & Orme, I. M. Progression of chronic pulmonary tuberculosis in mice aerogenically infected with virulent ***Mycobacterium*** tuberculosis. Tuber Lung Dis 78, 57-66 (1997).

DETD [0203] 30. Grandvalet, C., Servant, P. & Mazodier, P. Disruption of ***hspR*** , the repressor gene of the dnaK operon in *Streptomyces albus* G. Mol Microbiol 23, 77-84 (1997).

DETD [0204] 31. VanBogelen, R. A., Acton, M. A. & Neidhardt, F. C. Induction of the ***heat*** ***shock*** regulon does not produce thermotolerance in *Escherichia coli*. Genes Dev. 1, 525-531 (1987).

DETD . . . 32. Camacho, L. R., Ensergueix, D., Perez, E., Gicquel, B. & Guilhot, C. Identification of a virulence gene cluster of ***Mycobacterium*** tuberculosis by signature-tagged transposon mutagenesis. Mol Microbiol 34, 257-267 (1999).

DETD [0206] 33. Cox, J. S., Chen, B., McNeil, M. & Jacobs, W. R., Jr. Complex lipid determines tissue-specific replication of ***Mycobacterium*** tuberculosis in mice. Nature 402, 79-83 (1999).

DETD . . . 34. Manabe, Y. C., Saviola, B. J., Sun, L., Murphy, J. R. & Bishai, W. R. Attenuation of virulence in ***Mycobacterium*** tuberculosis expressing a constitutively active iron repressor. Proc Natl Acad Sci USA 96, 12844-12848 (1999).

DETD . . . J. S. & Jacobs, W. R. A novel mycolic acid cyclopropane synthetase is required for cording, persistence, and virulence of ***Mycobacterium*** tuberculosis. Molecular Cell 5, 717-727 (2000).

DETD [0209] 36. McKinney, J. D. et al. Persistence of ***Mycobacterium*** tuberculosis in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. Nature 406, 735-738 (2000).

DETD [0210] 37. Parrish, N. M., Dick, J. D. & Bishai, W. R. Mechanisms of latency in ***Mycobacterium*** tuberculosis. Trends Microbiol 6, 107-112 (1998).

DETD . . . Richmond, J. F. L., Suzue, K., Eisen, H. N. & Young, R. A. In vivo cytotoxic T lymphocyte elicitation by ***mycobacterial*** ***heat*** ***shock*** protein 70 fusion proteins maps to a discrete domain and is CD4.sup.+T cell independent. J. Exp Med 191, 403-408 (2000).

DETD [0215] 42. Mehlert, A. & Young, D. B. Biochemical and antigenic

characterization of the ***Mycobacterium*** tuberculosis 71kD antigen, a member of the 70kD ***heat*** - ***shock*** protein family. Mol Microbiol 3, 125-130 (1989).

DETD [0216] 43. Dussurget, O. et al. Role of ***Mycobacterium*** tuberculosis copper-zinc superoxide dismutase. Infect Immun 69, 529-533 (2001).

DETD . . . Duncan, K. & Young, D. B. Alteration of a single amino acid residue reverses fosfomycin resistance of recombinant MurA from ***Mycobacterium*** tuberculosis. Microbiology 145, 3177-3184 (1999).

DETD . . . Kurt-Jones, E. A., Stevenson, M. A., Chen, L. B., Finberg, R. W., Koo, G. C., and Calderwood, S. K. (2000) ***HSP70*** stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. Nat Med 6: 435-442.

DETD [0220] 47. Bucca, G., Brassington, A. M., Schonfeld, H. J., and Smith, C. P. (2000) The ***HspR*** regulon of streptomyces coelicolor: a role for the DnaK chaperone as a transcriptional corepressor. Mol Microbiol 38: 1093-1103.

DETD . . . P. E., Eichelberg, K., Mayhew, M., Rothman, J. E., Houghton, A. N., and Germain, R. N. (2000) Receptor-mediated uptake of Antigen/ ***Heat*** ***shock*** protein complexes results in major histocompatibility complex class I antigen presentation via two distinct processing pathways [In Process Citation]. J. . . .

DETD . . . Primm, T. P., Jakana, J., Lee, I. H., Serysheva, I., Chiu, W., Gilbert, H. F., and Quijcho, F. A. (1996) ***Mycobacterium*** tuberculosis 16-kDa antigen (***Hsp16*** .3) functions as an oligomeric structure in vitro to suppress thermal aggregation. J. Biol Chem 271: 7218-7223.

DETD . . . S., Hamlin, N., Holroyd, S., Hornsby, T., Jagels, K., Barrell, B. G., and et al. (1998) Deciphering the biology of ***Mycobacterium*** tuberculosis from the complete genome sequence [see comments] [published erratum appears in Nature 1998 Nov 12;396(6707):190]. Nature 393: 537-544.

DETD [0224] 51. Cunningham, A. F., and Spreadbury, C. L. (1998) ***Mycobacterial*** stationary phase induced by low oxygen tension: cell wall thickening and localization of the 16-kilodalton alphacrystallin homolog. J. Bacteriol 180: . . .

DETD [0225] 52. Fernandes, N. D., Wu, Q. L., Kong, D., Puyang, X., Garg, S., and Husson, R. N. (1999) A ***mycobacterial*** extracytoplasmic sigma factor involved in survival following ***heat*** ***shock*** and oxidative stress. J. Bacteriol 181: 4266-4274.

DETD . . . 53. Grandvalet, C., de Crecy-Lagard, V., and Mazodier, P. (1999) The ClpB ATPase of Streptomyces albus G belongs to the ***HspR*** ***heat*** ***shock*** regulon. Mol Microbiol 31:521-532.

DETD . . . Erickson, J. W., and Gross, C. A. (1984) The htpR gene product of E. coli is a sigma factor for ***heat*** - ***shock*** promoters. Cell 38: 383-390.

DETD [0229] 56. Hecker, M., Schumann, W., and Volker, U. (1996) ***Heat*** - ***shock*** and general stress response in Bacillus subtilis. Mol Microbiol 19: 417-428.

DETD . . . E., Kempse, K. E., Duncan, K., Stokes, R. W., Parish, T., and Stoker, N. G. (1999) Enhanced gene replacement in ***mycobacteria***. Microbiology 145:519-527.

DETD [0232] 59. Lee, B. Y., and Horwitz, M. A. (1995) Identification of macrophage and stress-induced proteins of ***Mycobacterium*** tuberculosis. J. Clin Invest 96: 245-249.

DETD . . . Manganelli, R., Dubnau, E., Tyagi, S., Kramer, F. R., and

- Smith, I. (1999) Differential expression of 10 sigma factor genes in
 Mycobacterium tuberculosis. Mol Microbiol 31: 715-724.
- DETD [0234] 61. Manganelli, R., Voskuil, M. I., Schoolnik, G. K., and Smith, I. (2001) The ***Mycobacterium*** tuberculosis ECF sigma factor sigmaE: role in global gene expression and survival in macrophages. Mol Microbiol 41: 423-437.
- DETD [0235] 62. Monahan, I., Betts, J., Banerjee, D., and Butcher, P. (2001) Differential expression of ***mycobacterial*** proteins following phagocytosis by macrophages. Microbiology 147:459-471.
- DETD [0236] 63. Narberhaus, F. (1999) Negative regulation of bacterial ***heat*** ***shock*** genes. Mol Microbiol 31:1-8.
- DETD [0237] 64. Parish, T., and Stoker, N. G. (1997) Development and use of a conditional antisense mutagenesis system in ***mycobacteria***. FEMS Microbiol Lett 154: 151-157.
- DETD [0238] 65. Patel, B. K., Banerjee, D. K., and Butcher, P. D. (1991) Characterization of the ***heat*** ***shock*** response in ***Mycobacterium*** bovis BCG. J. Bacteriol 173: 7982-7987.
- DETD . . . and Husson, R. N. (2001) The alternative sigma factor SigH regulates major components of oxidative and heat stress responses in ***Mycobacterium*** tuberculosis. J. Bacteriol 183: 6119-6125.
- DETD . . . Sherman, D. R., Voskuil, M., Schnappinger, D., Liao, R., Harrell, M. I., and Schoolnik, G. K. (2001) Regulation of the ***Mycobacterium*** tuberculosis hypoxic response gene encoding alpha-crystallin. Proc Natl Acad Sci U S A 98: 7534-7539.
- DETD . . . Hussell, T., Tormay, P., O'Gaora, P., Goyal, M., Betts, J., Brown, I. N., and Young, D. B. (2001) Overexpression of ***heat*** - ***shock*** proteins reduces survival of ***Mycobacterium*** tuberculosis in the chronic phase of infection. Nature Medicine 7: 732-737.
- DETD [0244] 71. Suto, R., and Srivastava, P. K. (1995) A mechanism for the specific immunogenicity of ***heat*** ***shock*** protein-chaperoned peptides. Science 269: 1585-1588.
- DETD [0245] 72. Wards, B. J., and Collins, D. M. (1996) Electroporation at elevated temperatures substantially improves transformation efficiency of slow-growing ***mycobacteria***. FEMS Microbiol Lett 145: 101-105.
- DETD . . . H., Imboden, P., Rane, S., Brown, P. O., and Schoolnik, G. K. (1999) Exploring drug-induced alterations in gene expression in ***Mycobacterium*** tuberculosis by microarray hybridization. Proc Natl Acad Sci U S A 96: 12833-12838.
- DETD [0247] 74. Wilson M., Voskuil M., Schnappinger D., Schoolnik GK (2001) Functional genomics of ***Mycobacterium*** tuberculosis using DNA microarrays in: Methods in Molecular Medicine, vol 54: ***Mycobacterium*** tuberculosis Protocols (eds: T. Parish & N. G. Stoker) Humana Press Inc, Totowa, N. J. pp335-357.
- DETD [0248] 75. Yang, H., Huang, S., Dai, H., Gong, Y., Zheng, C., and Chang, Z. (1999) The ***Mycobacterium*** tuberculosis small ***heat*** ***shock*** protein ***Hsp16*** .3 exposes hydrophobic surfaces at mild conditions: conformational flexibility and molecular chaperone activity. Protein Sci 8: 174-179.
- DETD [0249] 76. Young, D. B., and Garbe, T. R. (1991) ***Heat*** ***shock*** proteins and antigens of ***Mycobacterium*** tuberculosis. Infect J. Immun 59: 3086-3093.
- DETD [0250] 77. Yuan, Y., Crane, D. D., and Barry, C. E., 3rd (1996) Stationary phase-associated protein expression in ***Mycobacterium*** tuberculosis: function of the ***mycobacterial*** alphacrystallin homolog. J. Bacteriol 178: 4484-4492.

DETD . . . Q., Hickey, M. J., Sherman, D. R., and Barry, C. E., 3rd (1998)
The 16 kDa alpha-crystallin (Acr) protein of ***Mycobacterium***
tuberculosis is required for growth in macrophages. Proc Natl Acad Sci U
S A 95:9578-9583.

CLM What is claimed is:

1. An immunogenic composition comprising ***mycobacteria*** wherein
said ***mycobacteria*** comprises ***modified*** protein
production .

2. The composition of claim 1, wherein the modified protein expression
comprises an increase in ***heat*** ***shock*** protein
production.

3. The composition of claim 2, wherein the ***heat*** ***shock***
protein is selected from the group consisting of ***Hsp10*** ,
Hsp40 , ***Hsp60*** , ***Hsp70*** , ***Hsp90*** , GrpE,
ClpB and alpha-cystallin.

4. The composition of claim 1, wherein the ***mycobacteria*** is
selected from the group consisting of M. tuberculosis, M.
avium-intracellulare, M. bovis, M. kansasii, M. fortuitum, M. chelonae,
M. . . .

5. The composition of claim 1, wherein the ***mycobacteria***
comprises M. tuberculosis.

6. The composition of claim 5, wherein the ***heat*** ***shock***
protein comprises ***Hsp*** 60 or ***Hsp*** 70.

7. The composition of claim 5, wherein the ***heat*** ***shock***
protein consists of ***Hsp*** 60 and ***Hsp*** 70.

. . . human or animal comprising to said human or animal an immunogenic
composition wherein said composition comprises an pathogenic organism
having ***modified*** ***heat*** ***shock*** protein .
production .

11. The method of claim 10, wherein the pathogenic organism comprises M.
tuberculosis and the ***modified*** ***heat*** ***shock***
protein ***production*** comprises an increase in the production of
heat ***shock*** proteins.

12. The method of claim 11, wherein the ***heat*** ***shock***
protein is selected from the group consisting of ***Hsp10*** ,
Hsp40 , ***Hsp60*** , ***Hsp70*** , ***Hsp90*** , GrpE,
ClpB and alpha-cystallin.

13. The method of claim 11, wherein the ***heat*** ***shock***
proteins consists of ***Hsp*** 60 and ***Hsp*** 70.

14. A method for treating ***mycobacterial*** disease comprising
administering to a human or animal an immunogenic composition comprising
modified ***mycobacterial*** pathogens wherein said
mycobacterial pathogens have increased ***heat***
shock protein production.

15. The method of claim 14, wherein the ***mycobacterial*** disease
is selected from the group consisting of tuberculosis and Crohn's

disease.

16. The method of claim 15, wherein the ***heat*** ***shock*** protein is selected from the group consisting of ***Hsp10*** , ***Hsp40*** , ***Hsp60*** , ***Hsp70*** , ***Hsp90*** , GrpE, ClpB and alpha-cystallin.

17. The method of claim 15, wherein the ***heat*** ***shock*** protein consists of ***Hsp*** 60 and ***Hsp*** 70.

19. An immunogenic composition comprising an improved BCG vaccine wherein the vaccine comprises modified M. bovis having increased ***heat*** ***shock*** protein production.

20. The immunogenic composition of claim 19, wherein the ***heat*** ***shock*** protein is selected from the group consisting of ***Hsp10*** , ***Hsp40*** , ***Hsp60*** , ***Hsp70*** , ***Hsp90*** , GrpE, ClpB and alpha-cystallin.

L12 ANSWER 11 OF 14 USPATFULL on STN

AN 2002:272801 USPATFULL

TI Compositions and methods for the therapy and diagnosis of colon cancer

IN Stolk, John A., Bothell, WA, UNITED STATES

Xu, Jiangchun, Bellevue, WA, UNITED STATES

Chenault, Ruth A., Seattle, WA, UNITED STATES

Meagher, Madeleine Joy, Seattle, WA, UNITED STATES

PA Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)

PI US 2002150922 A1 20021017

AI US 2001-998598 A1 20011116 (9)

PRAI US 2001-304037P 20010710 (60)

US 2001-279670P 20010328 (60)

US 2001-267011P 20010206 (60)

US 2000-252222P 20001120 (60)

DT Utility

FS APPLICATION

LREP SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300, SEATTLE, WA, 98104-7092

CLMN Number of Claims: 17

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 9233

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compositions and methods for the therapy and diagnosis of cancer, particularly colon cancer, are disclosed. Illustrative compositions comprise one or more colon tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly colon cancer.

SUMM [2044] SEQ ID NO:1997 is the determined cDNA sequence for clone 62227174
R0394 :B12

DETD . . . 4

2598 Homo sapiens H2A histone
family, member Z (H2AFZ)
mRNA

R0369 H4 2.01

2594	Homo sapiens hypothetical protein (***HSPC236***), mRNA	R0363 E1	2.65
2604	Human proteasome (prosome, macropain) subunit, alpha type, 5	R0362 E12	2.03
2599	Homo sapiens S100 calcium-binding. . .	R0370 B6	2.44

L12 ANSWER 12 OF 14 USPATFULL on STN

AN 2002:242791 USPATFULL

TI Compositions and methods for the therapy and diagnosis of colon cancer

IN King, Gordon E., Shoreline, WA, UNITED STATES

Meagher, Madeleine Joy, Seattle, WA, UNITED STATES

Xu, Jiangchun, Bellevue, WA, UNITED STATES

Secrist, Heather, Seattle, WA, UNITED STATES

PA Corixa Corporation, Seattle, WA, UNITED STATES (U.S. corporation)

PI US 2002131971 A1 20020919

AI US 2001-33528 A1 20011226 (10)

RLI Continuation-in-part of Ser. No. US 2001-920300, filed on 31 Jul 2001, PENDING

PRAI US 2001-302051P 20010629 (60)

US 2001-279763P 20010328 (60)

US 2000-223283P 20000803 (60)

DT Utility.

FS APPLICATION

LREP SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300, SEATTLE, WA, 98104-7092

CLMN Number of Claims: 17

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 8083

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compositions and methods for the therapy and diagnosis of cancer, particularly colon cancer, are disclosed. Illustrative compositions comprise one or more colon tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly colon cancer.

SUMM [1978] In one preferred embodiment, the immunological fusion partner is derived from a ***Mycobacterium*** sp., such as a ***Mycobacterium*** tuberculosis-derived Ra12 fragment. Ra12 compositions and methods for their use in enhancing the expression and/or immunogenicity of heterologous polynucleotide/polypeptide sequences. . . incorporated herein by reference in its entirety. Briefly, Ra12 refers to a polynucleotide region that is a subsequence of a ***Mycobacterium*** tuberculosis MTB32A nucleic acid. MTB32A is a serine protease of 32 KD molecular weight encoded by a gene in virulent.

SUMM . . . which uses restriction enzymes to generate a fragment in the known region of the gene. The fragment is then circularized ***by*** intramolecular ligation ***and*** used as a template for PCR ***with*** ***divergent*** primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to ***a*** linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round. . .

DETD . . . fis, clone HEP14459,
highly similar to HUM3H3M Homo sapiens
3-hydroxy-
3-methylglutaryl coenzymeA synthase
SEQ ID NO: 1801 74815 Homo sapiens , ***heat*** ***shock***
40 kD protein 1, clone
MGC:8425, mRNA, complete cds
SEQ ID NO: 1802 74816 Homo sapiens hypothetical protein FLJ22195
(FLJ22195), mRNA
SEQ. . . 1806 74827 Homo sapiens ribophorin II, clone MGC: 1817,
mRNA,
complete cds
SEQ ID NO: 1807 74828 Homo sapiens similar to ***HSPC039***
protein (H. sapiens)
(LOC65818), mRNA
SEQ ID NO: 1808 74829 Homo sapiens cell cycle protein CDC20 mRNA,
complete cds
SEQ ID NO: . . . sapiens Alg5, S. cerevisiae, homolog of (ALG5),
mRNA
SEQ ID NO: 1819 74854 Human cis-acting sequence
SEQ ID NO: 1820 74856 Homo sapiens ***HSPC128*** protein (
HSPC128), mRNA
SEQ ID NO: 1821 74857 Homo sapiens cDNA FLJ11051 fis, clone
PLACE1004629, weakly similar to PROTEIN OS-9
PRECURSOR

SEQ ID NO: . . .

L12 ANSWER 13 OF 14 USPATFULL on STN

AN 2002:238647 USPATFULL

TI MHC conjugates useful in ameliorating autoimmunity

IN Clark, Brian R., Redwood City, CA, United States

Sharma, Somesh D., Los Altos, CA, United States

Lerch, Bernard L., Palo Alto, CA, United States

PA Anergen, Inc., Seattle, WA, United States (U.S. corporation)

PI US 6451314 B1 20020917

AI US 2000-602807 20000623 (9)

RLI Continuation of Ser. No. US 1995-462351, filed on 5 Jun 1995, now
patented, Pat. No. US 6106840 Division of Ser. No. US 1992-869293, filed
on 14 Apr 1992, now patented, Pat. No. US 5468481 Continuation-in-part
of Ser. No. US 1991-690840, filed on 23 Apr 1991, now patented, Pat. No.
US 5260422 Continuation-in-part of Ser. No. US 1990-576084, filed on 30
Aug 1990, now patented, Pat. No. US 5130297 Continuation of Ser. No. US
1988-210594, filed on 23 Jun 1988, now abandoned

DT Utility

FS GRANTED

EXNAM Primary Examiner: Saunders, David; Assistant Examiner: DeCloux, Amy

LREP Townsend and Townsend and Crew LLP

CLMN Number of Claims: 10

ECL Exemplary Claim: 1

DRWN 35 Drawing Figure(s); 26 Drawing Page(s)

LN.CNT 2474

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is directed to complexes consisting essentially of
an isolated MHC component and an autoantigenic peptide associated with
the antigen binding site of the MHC component. These complexes are
useful in treating autoimmune disease.

DETD . . . in pathogenesis have been characterized: in arthritis in rat

and mouse, native type-II collagen is identified in collagen-induced arthritis, and ***mycobacterial*** ***heat*** ***shock*** protein in adjuvant arthritis (Stuart et al. (1984), Ann. Rev. Immunol. 2:199-218; van Eden et al. (1988), Nature 331:171-173.); thyroglobulin.

DETD Down-regulation of RA by MHCII- ***HSP*** (180-188) Complexes
 DETD . . . the peptide by mixing 56 .mu.g of RT1B and 113 .mu.g of RT1D molecules with 50-fold molar excess of the ***heat*** ***shock*** protein (***HSP***) peptide, p(180-188), at 37.degree. C. for 48h in a total volume of 1 ml phosphate buffer pH 7.5 containing 1%. . .
 DETD Six male Lewis rats (age 77 days) were injected in both hind foot pads with 1 mg of ***Mycobacterium*** tuberculosis in incomplete Freund's adjuvant to induce arthritis. Three of the rats were treated with the MHC Class II+plus ***HSP180*** -188 complex intravenously on days 1, 4 and 7 after the induction of the disease. The other three rats were given. . .
 DETD . . . 0
 MHC alone 4 9.0 .+- 0.7* 3.25 .+- 0.5
 MHC + 5 7.88 .+- 1.4* 2.6 .+- 0.55
 HSP (180-188)
 Normal 5 5.55 .+- 0.36 00

*Statistically significant compared to saline treatment (p < 0.05 by student's t-test).

DETD . . . clinical stage 1 EAMG (approximately day 42-56 post-inoculation) were injected i.v. at five weekly intervals with saline, 25 .mu.g MHC II: ***HSP*** 180-188 (MHC II bearing an irrelevant ***heat*** ***shock*** peptide), 25 .mu.g MHC II alone (MHC II:0), or 5 .mu.g AChR.alpha. 100-116 alone (O:AChR.alpha. 100-116). The weight and clinical. . .
 DETD . . . rate in the control groups was 20% (16.7% saline, 0% Tc AChR.alpha. 100-116 alone, 20% MHC II alone, 20% MHC II: ***HSP*** 180-188). The time course of EAMG for representative rats in each treatment group is presented in Table 4.

DETD

TABLE 4

DAYS POST EAMG INDUCTION
 TREATMENT 61 123 224

MHC II:AChR.alpha. 100-116 2.5 0.0 0.0
 MHC II: ***HSP*** 180-188 3.0 3.0 Dead (day 138)
 MHC II:0 3.0 Dead (day 66)
 O:AChR 100-116 2.5 Dead (day 66)
 Saline 3.0 Dead (day 82)
 DETD . . . the MHC II:AChR.alpha. 100-116 treated rat shows improved mobility and posture, in contrast to the lone surviving rat treated with MHC: ***HSP*** 180-188.

DETD . . . 440 445

Gly

SEQUENCE CHARACTERISTICS:

LENGTH: 170 amino acids

TYPE: amino acid

STRANDEDNESS:

TOPOLOGY: linear

MOLECULE TYPE: protein

FEATURE:

NAME/KEY: Protein
LOCATION: 1..170
OTHER INFORMATION: /note= "myelin basic protein (MBP)"
FEATURE:
NAME/KEY: ***Modified*** -site
OTHER INFORMATION: / ***product*** = "OTHER" /note= "Xaa =
N-acetyl-alanine"
FEATURE:
NAME/KEY: ***Modified*** -site
OTHER INFORMATION: / ***product*** = "OTHER" /note= "Ala at
position 3 may be present or absent"
FEATURE:
NAME/KEY: Modified-site
LOCATION: 10
OTHER INFORMATION: /product= "OTHER" /note= "Xaa = Arg. . .
TYPE: DNA
SEQUENCE: 7
GACACCCCGT ACCTGGACAT CACCTACCAC TTCATCATGC AGCGTATCCC GCTGTACTTC 60
CTG 63
SEQUENCE CHARACTERISTICS:
LENGTH: 13 amino acids
TYPE: amino acid
STRANDEDNESS:
TOPOLOGY: linear
MOLECULE TYPE: peptide
FEATURE:
NAME/KEY: ***Modified*** -site
OTHER INFORMATION: / ***product*** = "OTHER" /note= "Xaa =
N-acetyl alanine"
SEQUENCE: 8
Xaa Ser Gln Lys Arg Pro Ser Gln Arg His Gly Ser Lys
1 5 10
SEQUENCE CHARACTERISTICS:
LENGTH: 12 amino acids
TYPE: amino acid
STRANDEDNESS:
TOPOLOGY: linear
MOLECULE TYPE: peptide
FEATURE:
NAME/KEY: ***Modified*** -site
OTHER INFORMATION: / ***product*** = "OTHER" /note= "Xaa =
N-acetyl phenylalanine"
FEATURE:
NAME/KEY: Modified-site
LOCATION: 12
OTHER INFORMATION: /product= "OTHER" /note= "Xaa = prolinamide"
SEQUENCE: 9
Xaa Phe Lys Asn Ile. . .

L12 ANSWER 14 OF 14 USPATFULL on STN

AN 2000:53875 USPATFULL

TI Method of identifying compounds affecting hedgehog cholesterol transfer

IN Beachy, Philip A., Baltimore, MD, United States

Porter, Jeffrey A., Belmont, MA, United States

PA The Johns Hopkins University School of Medicine, United States (U.S.
corporation)

PI US 6057091 20000502

AI US 1997-946329 19971007 (8)
 RLI Continuation-in-part of Ser. No. US 1996-729743, filed on 7 Oct 1996
 which is a continuation-in-part of Ser. No. US 1995-567357, filed on 4
 Dec 1995 which is a continuation-in-part of Ser. No. US 1994-349498,
 filed on 2 Dec 1994
 PRAI US 1997-61323P 19971002 (60)
 DT Utility
 FS Granted
 EXNAM Primary Examiner: Spector, Lorraine; Assistant Examiner: Kaufman, Claire
 M.
 LREP Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.
 CLMN Number of Claims: 4
 ECL Exemplary Claim: 1
 DRWN 126 Drawing Figure(s); 54 Drawing Page(s)
 LN.CNT 6997

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides two novel polypeptides, referred to as
 the "N" and "C" fragments of hedgehog, or N-terminal and C-terminal
 fragments, respectively, which are derived after specific cleavage at a
 G.sup..downarw. CF site recognized by the autoproteolytic domain in the
 native protein. Also included are sterol-modified hedgehog polypeptides
 and functional fragments thereof. Methods of identifying compositions
 which affect hedgehog activity based on inhibition of cholesterol
 modification of hedgehog protein are described.

DRWD FIG. 5 shows immunoblots showing ***heat*** ***shock*** induced
 expression of wild type and H329A mutant hh proteins in Drosophila
 embryos (A) and (B) are immunoblots developed using. . .

DRWD . . . as a .about.5-kDa species when cholesterol-modified. His.sub.6
 Hh-C.sub.17 was also incubated with 46 .mu.M [.sup.3 H]cholesterol/1 mM
 DTT, and no cholesterol- ***modified*** ***product*** was
 detected by autoradiography. A cholesterol-transfer activity 1% of
 wildtype could have been detected by this radioassay.

DRWD . . . addition of 50 mM DTT greatly increases the amount of cleavage
 products and addition of cholesterol does not produce a cholesterol-
 modified ***product*** (.about.5-kDa species). D303A was
 also incubated with 46 .mu.M [.sup.3 H]cholesterol/1 mM DTT, and no
 cholesterol- ***modified*** ***product*** was detected by
 autoradiography (data not shown). A cholesterol-transfer activity 1% of
 wildtype could have been detected by this radioassay.

DRWD . . . KLBA--predicted ATPase; HO--homothallic endonuclease. Species
 abbreviations: CAEEL--Caenorhabditis elegans; DANRE--Danio rerio;
 XENLA--Xenopus laevis; Cynpy--Cynops pyrrhogaster; DROHY--Drosophila
 hydei; DROME--Drosophila melanogaster; CANTR--Candida tropicalis;
 MYCLE-- ***Mycobacterium*** leprae; MYCXE-- ***Mycobacterium***
 xenopi; MYCTU-- ***Mycobacterium*** tuberculosis; PORPU--Porphyra
 purpurea; SYNSP--Synechocystis sp; CHLEU--Chlamydomonas;
 METJA--Methanococcus jannaschii; PYRFU--Pyrococcus furiosus;
 PYRSP--Pyrococcus sp.; THELI--Thermococcus litoralis. Several Hh and
 intein sequences closely. . .

DETD . . . as the small subunit of RUBISCO (Coruzzi, et al., EMBO J.,
 3:1671-1680, 1984; Broglie, et al., Science, 224:838, 1984); or
 heat ***shock*** promoters, e.g., soybean ***hsp17***
 .5-E or ***hsp17*** .3-B (Gurley, et al., Mol. Cell. Biol., 6:559,
 1986) may be used. These constructs can be introduced into plant cells
 using. . .

DETD . . . High level expression may also be achieved using inducible
 promoters, including, but not limited to, the metallothionine IIA

promoter and ***heat*** ***shock*** promoters.
 DETD . . . 368: 208, 1994). To ascertain the importance of
 auto-proteolysis for these functions, the H329A mutant gene under
 control of the ***hsp*** 70 promoter was introduced by P
 element-mediated transformation into the Drosophila germline. The hshh
 H329A construct was made identically to. . . stripes, embryos
 collected at 4 to 6 hours after egg laying (AEL) at 25.degree. C. were
 subjected to the following ***heat*** ***shock*** protocols
 prior to fixation. Embryos receiving single shocks (10 or 30 minutes at
 37.degree. C.) were allowed to recover for. . .
 DETD FIG. 5 shows that ***heat*** ***shock*** induction results in
 the formation of an abundant species that corresponds to U based on its
 mobility and its interaction. . .
 DETD . . . (FIG. 6, B and C; Table 1). The difference in efficiency ranges
 nearly as high as threefold depending upon the ***heat***
 shock regime, and these results suggest that auto-proteolysis
 of
 the Hh protein is important for optimal activity in embryonic signaling
 to. . .

DETD TABLE 1

Wild-type and mutant hh activity in embryonic induction of wg expression*			
	minutes of	***heat***	***shock***
10	30	10/10	30/30

hshh	1.0	+-	0.3 (93)
	1.5	+-	0.6 (120)
	2.9	+-	0.3 (41)
	2.8	+-	0.4 (54)

hshh. . .
 DETD . . . cell type when hh is expressed ubiquitously at high levels. We
 have reproduced suppression 3.degree. and some 4.degree. fates by
 heat ***shock*** induction of embryos that carry our
 wild-type construct (FIG. 6E), but find that the H329A mutant is unable
 to alter. . .
 DETD For studies of signaling in imaginal discs, a thermal cycler was
 utilized to subject larvae carrying ***heat*** ***shock***
 -inducible hh constructs to successive rounds of ***heat***
 shock and recovery. The effects of temperature cycling upon
 expression of dpp and wg in imaginal discs was examined by monitoring.
 . . contrast, discs from hshh H329A and control larvae showed very
 little change in wg and dpp expression, even under prolonged
 heat ***shock*** conditions and morphological changes were
 never observed. (M-O) The eye phenotypes of adult control (M), hshh (N)
 and hshh H329A. . .
 DETD . . . at least some activity in early embryonic and imaginal disc
 induction of wg and dpp expression; in contrast, even under ***heat***
 shock conditions far more severe than those required for
 effects
 by the wild-type protein, the H329A mutant remained completely inert
 with. . .

=> d bib ab kwic l13 1-

YOU HAVE REQUESTED DATA FROM 9 ANSWERS - CONTINUE? Y/(N):y

L13 ANSWER 1 OF 9 USPATFULL on STN

AN 2003:225302 USPATFULL
 TI Compositions and methods for treatment of neoplastic disease
 IN Terman, David S., Pebble Beach, CA, UNITED STATES
 PI US 2003157113 A1 20030821
 AI US 2000-751708 A1 20001228 (9)
 PRAI US 1999-173371P 19991228 (60)
 DT Utility
 FS APPLICATION
 LREP David S. Terman, P.O. Box 987, Pebble beach, CA, 93953
 CLMN Number of Claims: 60
 ECL Exemplary Claim: 1
 DRWN 3 Drawing Page(s)
 LN.CNT 15804

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention comprises compositions and methods for treating a tumor or neoplastic disease in a host, The methods employ conjugates comprising superantigen polypeptides, nucleic acids with other structures that preferentially bind to tumor cells and are capable of inducing apoptosis. Also provided are superantigen-glycolipid conjugates and vesicles that are loaded onto antigen presenting cells to activate both T cells and NKT cells. Cell-based vaccines comprise tumor cells engineered to express a superantigen along with glycolipids products which, when expressed, render the cells capable of eliciting an effective anti-tumor immune response in a mammal into which these cells are introduced. Included among these compositions are tumor cells, hybrid cells of tumor cells and accessory cells, preferably dendritic cells. Also provided are tumoricidal T cells and NKT cells devoid of inhibitory receptors or inhibitory signaling motifs which are hyperresponsive to the the above compositions and lipid-based tumor associated antigens that can be administered for adoptive immunotherapy of cancer and infectious diseases.

SUMM . . . cell both enterotoxins and --.alpha.-galactosylceramides are associated with numerous intracellular and membrane structures such as MHC, costimulatory and adhesion molecules, ***heat*** ***shock*** proteins, membrane glycolipids and glycosphingolipids which may improve immunogenicity and antigen presentation. They may also be transported in various vesicles. . . .

SUMM . . . structures may actually improve the T cell activating function of SAgS such as deoxyribonucleic acids, ribonucleic acids, tumor associated antigens, ***heat*** ***shock*** proteins, costimulatory molecules and adhesion molecules and endosomes. Cellular SAg peptides or nucleotides exist in association with tumor associated antigens, costimulants, adhesion molecules, ***heat*** ***shock*** proteins and MHC molecules, GPI-ceramides or SAg receptors (digalactosylceramides) which improve the immunogenicity of the tumor antigens. Therefore, these structural. . . .

SUMM . . . tumor cells, an immunogenic bacterial product such as Staphylococcal adhesin protein A, LPS, .beta.-glucans, and peptidoglycans, costimulatory and adhesion molecules, ***heat*** ***shock*** protein, growth factor receptors such as Her/neu and tumor markers such as PSA.

DETD [0074] 19. ***Heat*** - ***shock*** proteins, ATPases and G proteins

DETD . . . the ability to stimulate large subsets of T cells. SAgS include Staphylococcal enterotoxins, Streptococcal pyrogenic exotoxins, Mycoplasma antigens, rabies antigens, ***mycobacteria*** antigens,

EB viral antigens, minor lymphocyte stimulating antigen, mammary tumor virus antigen, ***heat*** ***shock*** proteins, stress peptides, clostridial and toxoplasmosis antigens and the like. Any SAg can be used as described herein, although, Staphylococcal. . .

DETD . . . in gram positive bacteria (such as Staphylococcus or Streptococcus), to nucleic acids encoding capsular polysaccharides and teichoic acids and in ***mycobacterial*** species to nucleic acids encoding lipoarabinan.

DETD . . . E. coli, Salmonella or Klebsiella or for bacteria which naturally produce lipoarabinomannans glycans or polysaccharides containing cell walls such as ***Mycobacterium*** and Streptococcus respectively. The SAg-polysaccharide constructs bind to CD1 receptors of antigen presenting cells. They are then capable of activating. . .

DETD . . . J et al., J. Natl. Cancer Inst. 72:955-962 (1984)). By synthesizing single stranded nucleotides corresponding to different regions in the ***Mycobacterium*** bovis genome, specific single stranded oligonucleotides that activate adherent splenocytes and enhanced natural killer cell activity have been identified. In. . .

DETD . . . response. Examples are LPS's of gram negative organisms, SAGs and peptidoglycans of gram positive organisms, fungal .beta.-glucans, bacterial glycosylceramides, and ***mycobacterial*** lipoarabinans. Numerous infectious agents with these structures cause potent immune reactions e.g. streptococcal cellulitis induced by S. pyogenes, E. coli.

DETD . . . molecule interact with different types of host cells. There is also evidence that immunopotentiating activity of a glycopeptide produced by ***mycobacteria*** is dependent on the saccharide residues of the molecule. The capsular polysaccharide of the Streptococcus is extremely immunogenic, consisting of. . .

DETD [0349] Genes Involved in ***Mycobacterial*** Cell Wall Biosynthesis

DETD . . . fused in frame or cotransfected into tumor cell with nucleic acids encoding the key enzymes involved in the biosynthesis of ***mycobacterial*** cell wall mycolic acid, phosphatidylinositol mannosides and lipoarabinans. A high affinity interaction of CD1b molecules with the acyl side chains. . .

DETD . . . in the biosynthesis of these molecules have been isolated. In addition to the usual fatty acids found in membrane lipids, ***mycobacteria*** have a wide variety of very long-chain saturated (C18-C32) and monounsaturated (up to C26) n-fatty acids. The occurrence of a-alkyl b-hydroxy very long chain fatty acids i e., mycolic acid is a hallmark of ***mycobacteria*** and related species.

Mycobacterial mycolic acids are the largest (C70-C90) with the largest-branch (C20-C25). The main chain contains one or two double bonds, cyclopropane. . . esterified to glycerol or sugars such as trehalose, glucose and fructose depending on the sugars present in the culture medium. ***Mycobacterium*** also contains several methyl-branched fatty acids. These include 10-methyl C18 fatty acid (tuberculostearic acid found esterified in phosphatidyl inositide mannosides),. . .

DETD [0355] The MAS gene encoding ***mycobacterial*** mycocerosic acid synthase is a dimer of the FAS gene. The cloning and sequencing of the MAS gene revealed the. . .

DETD . . . results in tissue specific forms of the protein, which can be intracellular, membrane bound, or secreted. In cells infected with ***mycobacteria***, the CD1 molecule binds and presents a ***mycobacterial*** membrane component, mycolic acid. Surface CD1 molecules present longer peptides than those normally found on class I

molecules. Whether CD1. . .

DETD . . . Nucleic acid encoding cell wall or cell membrane associated glycosylceramides or a branched, b hydroxy long-chain fatty acids found in ***mycobacteria*** and other bacteria are cotransfected into the CD1 transfected tumor cells. The tumor cell therefore displays glycosylceramides bound to the. . .

DETD [0460] 36. SAGs Combined with Signal Transduction Molecules or ***Heat*** ***Shock*** Proteins (HSPs)

DETD . . . frame to (or cotransfected with) a nucleic acid encoding "signal transduction molecules" such as Ras, JAK 1 and STAT-1a and ***heat*** ***shock*** proteins HSP-60, HSP-70, HSP-90a, HSP-90b, Cox-2 as well as heterotrimeric G proteins and ATPases. The genes for Staphylococcal HSP-70 useful. . .

DETD . . . limited to two recently discovered HSP genes, orf37 and orf 35 in Staphylococcus aureus that are upstream and downstream of ***grpE*** (hsp20), dnaK(hsp70) and dnaJ(hsp40) in the following sequence: orf37--hsp20--hsp70--hsp40--orf35. The promoters are located upstream of orf37 and upstream of hsp40. These. . .

DETD . . . pathway. Owing to the lower B-carotene content, Lp(a) may be more easily oxidized than LDL. Oxidized Lp(a) such as Lp(a) ***modified*** by malondialdehyde, a ***product*** generated in vivo from aggregated platelets, is avidly taken up by monocyte-macrophages. through the scavenger-receptor pathway. Lp(a) accumulates in either. . .

DETD . . . specifically to LBTAAs which include fatty acids, ceramides, glycolipids, sphingolipids, glycosphingolipids, phtosphingolipids, gangliosides, lipopeptides. IRIDAS recognize LBIDAS derived from bacteria, ***mycobacteria***, parasites, fungi, protozoans or plants and respond by producing an effective immunocyte response. These antigens comprise sphingolipids, glycopeptides, phytoglycolipids, mycoglycolipids,. . .

DETD . . . acids, ceramides, glycolipids, sphingolipids, glycosphingolipids, gangliosides, lipopeptides. Superantigens are also conjugated to LBIDAS, glycan and peptidoglycan antigens derived from bacteria, ***mycobacteria***, parasite, fungi or plants comprising sphingolipids, glycopeptides, peptidoglycans and teichoic acids, phytoglycolipids, mycoglycolipids, lipoarabinan, mycolic acids, Braun's lipopeptide, inositolphosphorylceramides and. . . given in Examples 15, 16, 21, 23, 53, 54. Conjugates consisting of SAg and LBIDAS derved from fungal, parasitic or ***mycobacterial*** sources are also useful for the treatment of infectious diseases such as tuberculosis, leishmaniasis, trypanosomiasis as given in Example 53.. . . a population of immunocytes with deleted (via gene knockout) or functionally inactivated (antisense) IRIDAS specific for bacterial, fungal, parasitic or ***mycobacterial*** antigens for use in adoptive immunotherapy of infectious disease (Examples 51, 52, 53).

DETD . . . 92:
1619-1623 (1995)

31. Lipid A biosynthetic (SEQ ID NOS: 105-112) Tumor
Schnaitman CA et al.,
genes lpxA-D
Microbiological Reviews 57:
655-682 (1993)

32. ***Mycobacterial*** mycolic acid (SEQ ID NOS: 113-114) Tumor
Fernandes ND et al., Gene
biosynthetic genes
170: 95-99 (1996); Mathur M

et al., J.Biol.. . .

DETD . . . and greatly restricted anchors are preferred. This recognition of CD1-presented antigens depends on the type and distribution of sugar residues. ***Mycobacterial*** cell wall antigens namely mycolic acids and lipoarabinomannan also bind to CD1. Recently several glycosylceramides, in particular, monogalactosyl ceramides GalCer). .

DETD . . . or exosomes comprising SAg-GalCer complexes or SAg-tumor peptide (including but not limited to normal mutated structures). The ternary complexes of SAg-GalCer- ***heat*** ***shock*** protein and tumor peptide- ***heat*** ***shock*** protein are also useful. These complexes may be in or soluble or immobilized form, attached to a CD1 or MHC. . .

DETD . . . acid and growth factor receptor nucleic acids

18. SAg-encoding nucleic acid and cell cycle protein nucleic acids

19. SAg-encoding nucleic acid and ***heat*** ***shock*** protein nucleic acids

20. SAg-encoding nucleic acid and chemokine nucleic acids

21. SAg-encoding nucleic acid and cytokine nucleic acids

22. SAg-encoding nucleic acid. . .

DETD . . . molecules containing amino acid sequences and homologous to the enterotoxin family of molecules. To this extent, mammary tumor virus sequences, ***heat*** ***shock*** proteins, stress peptides, Mycoplasma and ***mycobacterial*** antigens, and minor lymphocyte stimulating loci bearing tumoricidal structural homology to the enterotoxin family are useful as anti-tumor agents. Hybrid. . .

DETD . . . commonly used inducible promoters include the metallothionein CUP1 promoter, which is tightly controlled by copper; promoters activated in response to ***heat*** ***shock***, which are of particular interest for expression in the temperature-sensitive sec6-4 mutant and the PH05 promoter, which is derepressed at. . .

DETD [1886] C57 BL/6 mice are used. These mice are natural-killer-cell-deficient. Beige mice are infected with many of the nontuberculous ***mycobacteria*** : MAC, M. kansasii, M. simiac, M. malmoense and M. genavense. Same-sex mice 5-7 weeks old are allowed to acclimate for. .

DETD [1888] Primary cultures of MAC (M. kansasii or other ***mycobacteria***) to be used for infection are obtained from clinical isolates of patients with disseminated MAC infection, or the American Type. . .

DETD [1955] Preparation of Lipid-Based Tumor Associated Antigens (LBTAAs) & Lipid-Based Infectious Disease Associated Antigens (LBIDAs) of Bacterial, Fungal, Yeast, Parasitic, ***Mycobacterial***, Invertebrate and Protozoan Origin

CLM What is claimed is:

2 The receptor of claim 1 wherein the lipid antigen is a bacterial, fungal, protozoal or ***mycobacterial*** antigen.

. . . cell wherein said receptor inhibits cellular activation by receptors specific for lipid-based infectious disease associated antigens derived from bacteria, fungi, ***mycobacterium***, parasite, virus, eukaryote or prokaryote antigens in the context of MHC or CD1.

11. The lipid antigens derived from bacteria, ***mycobacteria***, fungi and protozoa marine invertebrates of claim 2 wherein said lipid antigens are selected from the group consisting of glycosylceramides, .

. . . based inhibitory motifs which inhibits cellular activation by receptors specific for lipid-based infectious disease associated antigens derived from bacteria, fungi, ***mycobacteria***, parasite, virus, eukaryote or prokaryote antigens are deleted or functionally deactivated.

. . . of claims 24-29 wherein said superantigen comprises a staphylococcal enterotoxin, a streptococcal pyrogenic exotoxin, mycoplasma arthritides, rabies virus, clostridial antigen, ***heat*** ***shock*** protein.

L13 ANSWER 2 OF 9 USPATFULL on STN

AN 2003:152692 USPATFULL

TI Diagnosis methods based on microcompetition for a limiting GABP complex

IN Polansky, Hanan, Rochester, NY, UNITED STATES

PI US 2003104358 A1 20030605

AI US 2002-219649 A1 20020815 (10)

RLI Continuation-in-part of Ser. No. US 2000-732360, filed on 7 Dec 2000, PENDING

DT Utility

FS APPLICATION

LREP Hanan Polansky, 3159 S. Winton Rd., Rochester, NY, 14623

CLMN Number of Claims: 32

ECL Exemplary Claim: 1

DRWN 28 Drawing Page(s)

LN.CNT 14430

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Microcompetition for GABP between a foreign polynucleotide and cellular GABP regulated genes is a risk factor associated with many chronic diseases such as obesity, cancer, atherosclerosis, stroke, osteoarthritis, diabetes, asthma, and other autoimmune diseases. The invention uses this novel discovery to present assays for the diagnosis of these chronic diseases. The assays are based on measuring the cellular copy number of the foreign polynucleotide, measuring the rate of complex formation between GABP and either the foreign polynucleotide, or a cellular GABP regulated gene, identifying modified expression of a cellular GABP regulated gene, or identifying modified activity of the gene product of a GABP regulated gene. The invention also presents other foreign polynucleotide-type assays.

DETD . . . LPL, ExoKII, FAS, TSP-1, FGF-4, .alpha.1-chim, Tr Hydr, NaKATPsea-3, PDFG.beta., FerH, MHC IA2 B8, Cw2Ld and B7, MDR1, CYP1A1, c-JUN, ***Grp78***, Hsp70, ADH2, GPAT, FPP, HMG, HSS, SREBP2, GHR, CP2, .beta.-actin, TK, TopoII.alpha., I, II, III, IV, cdc25, cdc2, cyclA, cyclB1, . . .

DETD . . . DNA sample, one using primers specific for the DNA prior to bisulfite treatment, and one using primers for the chemically ***modified*** DNA. The amplification ***products*** are resolved on native polyacrylamide gels and visualized by staining with ethidium bromide followed by UV illumination. Amplification products detected. .

DETD . . . islets compared with various other mouse tissues (Lenzen 1996.sup.548). Moreover, induction of cellular stress by high glucose, high oxygen, and ***heat*** ***shock*** treatment did not affect antioxidant enzyme expression in rat pancreatic islets or in RINm5F insulin-producing cells (Tiedge 1997.sup.549). Based on. . .

DETD . . . copy number of viral genome in the subsequent latent period.

Infection with some viruses, such as measles, hepatitis A, and
 Mycobacterium tuberculosis induce a strong polarized Th1-type
 response in early life. These infections reduce GABP virus replication
 and subsequent genome copy. . . .

DETD [2078] BCG is a freeze-dried preparation made from a living culture of
 the Calmette-Guerin strain of ***mycobacterium*** Bovis. It was
 first developed as a vaccine against tuberculosis in 1921 but also has
 been used as an immunotherapeutic. . . .

DETD [2079] Results of numerous studies suggest that measles, hepatitis A,
 and ***Mycobacterium*** tuberculosis infection in early life may
 prevent subsequent development of atopic diseases. In humans,
 immunomodulation during the first two years. . . .

DETD [2081] Another study showed that an infection of NOD mice with
 Mycobacterium avium, before the mice show overt diabetes,
 results in permanent protection of the animals from diabetes. This
 protective effect was. . . .

DETD . . . G N, Dykstra J, Roberts E M, Jayanti V K, Hickman D, Uchic J,
 Yao Y, Surber B, Thomas S,
 Granneman ***GRPotent*** inhibition of the cytochrome P-450 3A-mediated
 human liver microsomal
 metabolism of a novel HIV protease inhibitor by ritonavir: A positive
 drug-drug. . . .

DETD . . . T, Bennett S, Wheeler
 J, Huygen K, Aaby P, McAdam K P, Newport M J. Newborns develop a Th1-type
 immune response to
 Mycobacterium bovis bacillus Calmette-Guerin vaccination. J Immunol.
 1999 Aug 15; 163(4): 2249-55.

.sup.776 Starr S E, Visintine A M, Tomeh M O,. . . . of symptoms of asthma,
 rhinitis, and eczema. Thorax 2000
 Jun; 55(6): 449-53.

.sup.780 von Hertzen L, Klaukka T, Mattila H, Haahtela T. ***Mycobacterium***
 tuberculosis infection and the
 subsequent development of asthma and allergic conditions. J Allergy Clin
 Immunol. 1999
 Dec; 104(6): 1211-4.

.sup.781 Scanga C B,. . . . 1 diabetes mellitus: is there a link? Drug Saf.
 1999 Mar; 20(3): 207-12.

.sup.786 Martins T C, Aguas A P. Mechanisms of ***Mycobacterium***
 avium-induced resistance against insulin-
 dependent diabetes mellitus (IDDM) in non-obese diabetic (NOD) mice: role of
 Fas and Th1 cells. Clin
 Exp Immunol 1999 Feb; 115(2): 248-54.

.sup.787 Bras A, Aguas A P. Diabetes-prone NOD mice are resistant to
 Mycobacterium avium and the infection
 prevents autoimmune disease. Immunology. 1996 Sep; 89(1): 20-5.

.sup.788 Pabst H F, Spady D W, Pilarski L M,. . . .

L13 ANSWER 3 OF 9 USPATFULL on STN
 AN 2003:106233 USPATFULL
 TI Compositions and methods for the therapy and diagnosis of pancreatic
 cancer
 IN Benson, Darin R., Seattle, WA, UNITED STATES
 Kalos, Michael D., Seattle, WA, UNITED STATES
 Lodes, Michael J., Seattle, WA, UNITED STATES
 Persing, David H., Redmond, WA, UNITED STATES
 Hepler, William T., Seattle, WA, UNITED STATES
 Jiang, Yuqiu, Kent, WA, UNITED STATES

PA Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)

PI US 2003073144 A1 20030417

AI US 2002-60036 A1 20020130 (10)

PRAI US 2001-333626P 20011127 (60)

US 2001-305484P 20010712 (60)

US 2001-265305P 20010130 (60)

US 2001-267568P 20010209 (60)

US 2001-313999P 20010820 (60)

US 2001-291631P 20010516 (60)

US 2001-287112P 20010428 (60)

US 2001-278651P 20010321 (60)

US 2001-265682P 20010131 (60)

DT Utility

FS APPLICATION

LREP SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300,
SEATTLE, WA, 98104-7092

CLMN Number of Claims: 17

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 14253

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compositions and methods for the therapy and diagnosis of cancer,
particularly pancreatic cancer, are disclosed. Illustrative compositions
comprise one or more pancreatic tumor polypeptides, immunogenic portions
thereof, polynucleotides that encode such polypeptides, antigen
presenting cell that expresses such polypeptides, and T cells that are
specific for cells expressing such polypeptides. The disclosed
compositions are useful, for example, in the diagnosis, prevention
and/or treatment of diseases, particularly pancreatic cancer.

SUMM [2043] SEQ ID NO:2003 is the determined cDNA sequence of clone 61496359

DETD . . . and Ubiq.-

270	PNCM-86	72179	binding domains Hu. Protein A kinase (PRKA) anchor protein
271, 272	PNCM-87	73421	(gravin) 12 (AKAP12) Hu. ***heat*** ***shock***
273	105kD, antigen NY-CO-25 PNCM-88	72180	Hu. ***heat*** ***shock***
274	105kD, antigen NY-CO-25 (Colon PNCM-89	72181	cancer Ag.) Hu. ferritin, heavy polypeptide 1
275	(FTH1) PNCM-90	72182	Hu. frizzled (Drosophila) homolog.
	PNCM-95	72187	Hu. kinectin 1 (kinesin receptor) (KTN1) [bp 813- 1223]
281	PNCM-96	72188	Hu. prosaposin [bp 608-1018]
282	PNCM-97	72189	Hu. ***heat*** ***shock***
	105kD . . . [bp 1-412]		
283	PNCM-98	72190	Hu. clone IMAGE:3449323
284	PNCM-99	72191	Hu. rabaptin-5 [bp 1578-1990]
285	PNCM-100	72192	Hu. . . . fis, clone LNG01826
298	PNCM-119	72205	Hu. cDNA DKFZp586F1918
299, 300	PNCM-120	72206	Macaca fascicularis brain cDNA, clone Qf1A-11332
301	PNCM-122	73422	Hu. ***heat*** ***shock***

302	105kD, antigen NY-CO-25 PNCM-123 helicase	73423	Hu. IMAGE:3355762, chromodomain DNA binding protein 1-like
303	PNCM-124 74602 Hu. fer-1 (C. elegans)-like 3 (myoferlin) (FER1L3)	73424	Hu. kinectin 1 (kinesin receptor).
337	PNCM-148 activator	73445	Hu. prosaposin (PSAP), sphingolipid protein 1
338	PNCM-150 105kD, antigen NY-CO-25	73456	Hu. ***heat*** ***shock***
339	PNCM-151 105kD (HSP-105B)	73585	Hu. ***heat*** ***shock***
340, 341	PNCM-152 protein	73586	Hu. Protein A kinase (PRKA) anchor (gravin) 12
342, 343	PNCM-153 inhibitor (GABA receptor protein)	73587	Hu. cleavage stimulation. modulator, acyl-Coenzyme A binding
394, 395	PNCM-193	74632	Hu. endozepine, vimentin
396	PNCM-202 ***GRP58***)	77105	Hu. glucose-regulated protein, 58kD (
397	PNCM-208	77108	Hu. rabaptin-5 (RAB5EP)
398	PNCM-210	77109	Hu. vimentin (VIM)
399	PNCM-215 clone	77114	Hu. hypothetical protein FLJ10634, MGC:944
400	PNCM-219	77118.	

L13 ANSWER 4 OF 9 USPATFULL on STN

AN 2003:100088 USPATFULL

TI Treatment methods based on microcompetition for a limiting GABP complex

IN Polansky, Hanan, Rochester, NY, UNITED STATES

PI US 2003069199 A1 20030410

AI US 2002-219334 A1 20020815 (10)

RLI Continuation-in-part of Ser. No. US 2000-732360, filed on 7 Dec 2000,
PENDING

DT Utility

FS APPLICATION

LREP Hanan Polansky, 3159 S. Winton Rd., Rochester, NY, 14623

CLMN Number of Claims: 26

ECL Exemplary Claim: 1

DRWN 28 Drawing Page(s)

LN.CNT 14837

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Microcompetition for GABP between a foreign polynucleotide and a cellular GABP regulated gene is a risk factor associated with chronic disease such as obesity, cancer, atherosclerosis, stroke, osteoarthritis, diabetes, asthma, and other autoimmune diseases. The invention uses this novel discovery to present methods for the treatment of these chronic diseases. The methods are based on modifying such microcompetition, or the effect of such microcompetition on the cell. For instance, treatment may modify the cellular copy number of the foreign polynucleotide, change the rate of complex formation between GABP and either the foreign polynucleotide or the cellular GABP regulated gene, vary the expression of the cellular GABP regulated gene,

or manipulate the activity of the gene product of the cellular GABP regulated gene. The invention also presents methods for treatment of chronic diseases resulting from other foreign polynucleotide-type disruptions.

DETD . . . LPL, ExoKII, FAS, TSP-1, FGF-4, .alpha.1-chim, Tr Hydr, NaKATPsea-3, PDFG.beta., FerH, MHC IA2 B8, Cw2Ld and B7, MDR1, CYP1A1, c-JUN, ***Grp78***, Hsp70, ADH2, GPAT, FPP, HMG, HSS, SREBP2, GHR, CP2, .beta.-actin, TK, TopoII.alpha., I, II, III, IV, cdc25, cdc2, cyclA, cyclB1, . . .

DETD . . . DNA sample, one using primers specific for the DNA prior to bisulfite treatment, and one using primers for the chemically ***modified*** DNA. The amplification ***products*** are resolved on native polyacrylamide gels and visualized by staining with ethidium bromide followed by UV illumination. Amplification products detected. .

DETD . . . islets compared with various other mouse tissues (Lenzen 1996.sup.548). Moreover, induction of cellular stress by high glucose, high oxygen, and ***heat*** ***shock*** treatment did not affect antioxidant enzyme expression in rat pancreatic islets or in RINm5F insulin-producing cells (Tiedge 1997.sup.549). Based on. . .

DETD . . . copy number of viral genome in the subsequent latent period. Infection with some viruses, such as measles, hepatitis A, and ***Mycobacterium*** tuberculosis induce a strong polarized Th1-type response in early life. These infections reduce GABP virus replication and subsequent genome copy. . .

DETD [2107] BCG is a freeze-dried preparation made from a living culture of the Calmette-Guerin strain of ***mycobacterium*** Bovis. It was first developed as a vaccine against tuberculosis in 1921 but also has been used as an immunotherapeutic. . .

DETD [2108] Results of numerous studies suggest that measles, hepatitis A, and ***Mycobacterium*** tuberculosis infection in early life may prevent subsequent development of atopic diseases. In humans, immunomodulation during the first two years. . .

DETD [2110] Another study showed that an infection of NOD mice with ***Mycobacterium*** avium, before the mice show overt diabetes, results in permanent protection of the animals from diabetes. This protective effect was. . .

DETD . . . N, Dykstra J, Roberts E M, Jayanti V K, Hickman D, Uchic J, Yao Y, Surber B, Thomas S, Granneman ***GRPotent*** inhibition of the cytochrome P-450 3A-mediated human liver microsomal metabolism of a novel HIV protease inhibitor by ritonavir: A positive. . .

DETD . . . S, Wheeler J, Huygen K, Aaby P, McAdam K P, Newport M J. Newborns develop a Th1-type immune response to ***Mycobacterium*** bovis bacillus Calmette-Guerin vaccination. J Immunol. 1999 Aug 15;163(4):2249-55.

DETD [2897] .sup.780 von Hertzen L, Klaukka T, Mattila H, Haahtela T. ***Mycobacterium*** tuberculosis infection and the subsequent development of asthma and allergic conditions. J Allergy Clin Immunol. 1999 Dec; 104(6):1211-4.

DETD [2903] .sup.786 Martins T C, Aguas A P. Mechanisms of ***Mycobacterium*** avium-induced resistance against insulin-dependent

diabetes mellitus (IDDM) in non-obese diabetic (NOD) mice: role of Fas and Th1 cells. Clin Exp. . .

DETD [2904] .sup.787 Bras A, Aguas A P. Diabetes-prone NOD mice are resistant to ***Mycobacterium*** avium and the infection prevents autoimmune disease. Immunology. 1996 Sep;89(1):20-5.

L13 ANSWER 5 OF 9 USPATFULL on STN

AN 2003:99511 USPATFULL

TI Drug discovery assays based on microcompetition for a limiting GABP complex

IN Polansky, Hanan, Rochester, NY, UNITED STATES

PI US 2003068616 A1 20030410

AI US 2002-223050 A1 20020814 (10)

RLI Continuation-in-part of Ser. No. US 2000-732360, filed on 7 Dec 2000, PENDING

DT Utility

FS APPLICATION

LREP Hanan Polansky, 3159 S. Winton Rd., Rochester, NY, 14623

CLMN Number of Claims: 55

ECL Exemplary Claim: 1

DRWN 28 Drawing Page(s)

LN.CNT 14981

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A recent discovery showed that microcompetition for GABP between a foreign polynucleotide and a cellular GABP regulated gene is a risk factor for some of the major chronic diseases, such as obesity, cancer, atherosclerosis, stroke, osteoarthritis, diabetes, asthma, and other autoimmune diseases. The invention uses this novel discovery to present assays for screening compounds based on their effectiveness in modulating such microcompetition, or the effects of such microcompetition on the cell. The selected compounds can be used in treatment of these chronic diseases. The invention also presents assays for screening compounds that can be used in treatment of chronic diseases resulting from other foreign polynucleotide-type disruptions.

DETD . . . LPL, ExoKII, FAS, TSP-1, FGF-4, .alpha.1-chim, Tr Hydr, NaKATPsea-3, PDFG.beta., FerH, MHC IA2 B8, Cw2Ld and B7, MDR1, CYP1A1, c-JUN, ***Grp78***, Hsp70, ADH2, GPAT, FPP, HMG, HSS, SREBP2, GHR, CP2, .beta.-actin, TK, TopoII.alpha., I, III, III, IV, cdc25, cdc2, cyclA, cyclB1, . . .

DETD . . . DNA sample, one using primers specific for the DNA prior to bisulfite treatment, and one using primers for the chemically ***modified*** DNA. The amplification ***products*** are resolved on native polyacrylamide gels and visualized by staining with ethidium bromide followed by UV illumination. Amplification products detected.

DETD . . . islets compared with various other mouse tissues (Lenzen 1996.sup.548). Moreover, induction of cellular stress by high glucose, high oxygen, and ***heat*** ***shock*** treatment did not affect antioxidant enzyme expression in rat pancreatic islets or in RINm5F insulin-producing cells (Tiedge 1997.sup.549). Based on. . .

DETD . . . copy number of viral genome in the subsequent latent period. Infection with some viruses, such as measles, hepatitis A, and ***Mycobacterium*** tuberculosis induce a strong polarized Th1-type response in early life. These infections reduce GABP virus replication and subsequent genome copy. . . .

DETD [2087] BCG is a freeze-dried preparation made from a living culture of the Calmette-Guerin strain of ***mycobacterium*** Bovis. It was first developed as a vaccine against tuberculosis in 1921 but also has been used as an immunotherapeutic. . . .

DETD [2088] Results of numerous studies suggest that measles, hepatitis A, and ***Mycobacterium*** tuberculosis infection in early life may prevent subsequent development of atopic diseases. In humans,

immunomodulation during the first two years. . . .

DETD [2090] Another study showed that an infection of NOD mice with
 Mycobacterium avium, before the mice show overt diabetes,
 results in permanent protection of the animals from diabetes. This
 protective effect was. . . .

DETD . . . N, Dykstra J, Roberts E M, Jayanti V K, Hickman D, Uchic J, Yao
 Y, Surber B, Thomas S, Granneman ***GRPotent*** inhibition of the
 cytochrome P-450 3A-mediated human liver microsomal metabolism of a
 novel HIV protease inhibitor by ritonavir: A positive. . . .

DETD . . . Wheeler J, Huygen K, Aaby P, McAdam K P, Newport M J. Newborns
 develop a Th 1-type immune response to ***Mycobacterium*** bovis
 bacillus Calmette-Guerin vaccination. J. Immunol. Aug. 15,
 1999;163(4):2249-55.

DETD [2877] .sup.780 von Hertzen L, Klaukka T, Mattila H, Haahtela T.
 Mycobacterium tuberculosis infection and the subsequent
 development of asthma and allergic conditions. J Allergy Clin Immunol.
 1999 December;104(6):1211-4.

DETD [2883] .sup.786 Martins T C, Aguas A P. Mechanisms of
 Mycobacterium avium-induced resistance against insulin-
 dependent
 diabetes mellitus (IDDM) in non-obese diabetic (NOD) mice: role of Fas
 and Th1 cells. Clin Exp. . . .

DETD [2884] .sup.787 Bras A, Aguas A P. Diabetes-prone NOD mice are resistant
 to ***Mycobacterium*** avium and the infection prevents autoimmune
 disease. Immunology. 1996 September;89(1):20-5.

L13 ANSWER 6 OF 9 USPATFULL on STN

AN 2003:40533 USPATFULL

TI Methods for the inhibition of epstein-barr virus transmission employing
 anti-viral peptides capable of abrogating viral fusion and transmission

IN Barney, Shawn O'Lin, Cary, NC, United States

Lambert, Dennis Michael, Cary, NC, United States

Petteway, Stephen Robert, Cary, NC, United States

PA Trimeris, Inc., Durham, NC, United States (U.S. corporation)

PI US 6518013 B1 20030211

AI US 1995-485546 19950607 (8)

RLI Continuation-in-part of Ser. No. US 1994-360107, filed on 20 Dec 1994,
 now patented, Pat. No. US 6017536 Continuation-in-part of Ser. No. US
 1994-255208, filed on 7 Jun 1994 Continuation-in-part of Ser. No. US
 1993-73028, filed on 7 Jun 1993, now patented, Pat. No. US 5464933

DT Utility

FS GRANTED

EXNAM Primary Examiner: Scheiner, Laurie; Assistant Examiner: Parkin, Jeffrey
 S.

LREP Pennie & Edmonds LLP, Nelson, M. Bud

CLMN Number of Claims: 22

ECL Exemplary Claim: 1

DRWN 84 Drawing Figure(s); 83 Drawing Page(s)

LN.CNT 24700

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Fusion of the viral envelope, or infected cell membranes with uninfected
 cell membranes, is an essential step in the viral life cycle. Recent
 studies involving the human immunodeficiency virus type 1(HIV-1)
 demonstrated that synthetic peptides (designated DP-107 and DP-178)
 derived from potential helical regions of the transmembrane (TM)
 protein, gp41, were potent inhibitors of viral fusion and infection. A
 computerized antiviral searching technology (C.A.S.T.) that detects

related structural motifs (e.g., ALLMOTI 5, 107.times.178.times.4, and PLZIP) in other viral proteins was employed to identify similar regions in the Epstein-Barr virus (EBV). Several conserved heptad repeat domains that are predicted to form coiled-coil structures with antiviral activity were identified in the EBV genome. Synthetic peptides of 16 to 39 amino acids derived from these regions were prepared and their antiviral activities assessed in a suitable in vitro screening assay. These peptides proved to be potent inhibitors of EBV fusion. Based upon their structural and functional equivalence to the known HIV-1 inhibitors DP-107 and DP-178, these peptides should provide a novel approach to the development of targeted therapies for the treatment of EBV infections.

DETD . . . HAEMOPHILUS DUCREYI 339-366 417-444
PCH60_LEGMI 60 KD CHAPERONIN LEGIONELLA MICDADEI 299-333
PCH60_LEGPN 60 KD CHAPERONIN LEGIONELLA PNEUMOPHILA 298-332 452-479
PCH60_MYCLE 60 KD CHAPERONIN ***MYCOBACTERIUM*** LEPRAE 125-152 236-263
337-364
PCH60_MYCTU 60 KD CHAPERONIN ***MYCOBACTERIUM*** TUBERCULOSIS 125-152
337-364
& BOVIS
PCH60_PSEAE 60 KD CHAPERONIN PSEUDOMONAS AERUGINOSA 339-366
PCH60_RHILV 60 KD CHAPERONIN RHIZOBIUM LEGUMINOSARUM 117-163 322-370 425-466
PCH60_RICTS 60. . . 250-277
MINANT PROTEIN
PCLD_SALTY CHAIN LENGTH DETER- SALMONELLA TYPHIMURIUM 96-127 151-212
MINANT PROTEIN
PCLOS_CLOHI ALPHA-CLOSTRIPAIN CLOSTRIDIUM HISTOLYTICUM 30-58 497-524
PRECURSOR
PCLPA_ECOLI ATP-BINDING SUBUNIT ***CLPA*** ESCHERICHIA COLI 655-695
PCLPA_RHOBL ***CLPA*** HOMOLOG PROTEIN RHODOPSEUDOMONAS BLASTICA 439-466
PCLPB_BACNO ***CLPB*** HOMOLOG PROTEIN BACTEROIDES NODOSUS 116-157
442-476 558-595
PCLPB_ECOLI ***CLPB*** PROTEIN ESCHERICHIA COLI 444-489 563-590
PCLPX_AZOVI ***CLPX*** HOMOLOG PROTEIN AZOTOBACTER VINELANDII 215-242
332-359
PCLPX_ECOLI ATP-BINDING SUBUNIT ***CLPX*** ESCHERICHIA COLI 255-282
PCN16_ECOLI 2',3'-CYCLIC-NUC 2'- ESCHERICHIA COLI 50-77
PHOSPHODIESTERASE
PRECURS
PCODA_ECOLI CYTOSINE DEAMINASE ESCHERICHIA COLI 102-129
PCOM1_BACSU A COMPETENCE PROTEIN 1 BACILLUS. . . CAULOBACTER CRESCENTUS
561-588
PDNAK_CLOAB DNAK PROTEIN CLOSTRIDIUM ACETOBUTYLICUM 499-526
PDNAK_CLOPE DNAK PROTEIN CLOSTRIDIUM PERFRINGENS 496-527
PDNAK_METMA DNAK PROTEIN METHANOSARCINA MAZEI 523-550
PDNAK_MYCTU DNAK PROTEIN ***MYCOBACTERIUM*** TUBERCULOSIS 502-529
PDNAK_STRCO DNAK PROTEIN STREPTOMYCES COELICOLOR 45-72 533-572
PDNIR_ECOLI REGULATORY PROTEIN DNIR ESCHERICHIA COLI 114-141
PDNLI_ZYMMO DNA LIGASE ZYMOMONAS MOBILIS 658-712
PDNRJ_STRPE TRANSDUCTION. . .
DETD . . . THERMOPLASMA ACIDOPHILUM 13-40 49-76 220-247
PEFG_ANANI ELONGATION FACTOR G ANACYSTIS NIDULANS 332-359
PEFG_ECOLI ELONGATION FACTOR G ESCHERICHIA COLI 234-261
PEFG_MYCLE ELONGATION FACTOR G ***MYCOBACTERIUM*** LEPRAE 211-259 330-357
PEFG_SALTY ELONGATION FACTOR G SALMONELLA TYPHIMURIUM 234-261
PEFG_SPIPL ELONGATION FACTOR G SPIRULINA PLATENSIS 334-374 481-511
PEFG_SYNY3 ELONGATION FACTOR G SYNECHOCYSTIS. . . FACTOR TU HALOARCUA

MARISMORTUI 4-31

PEFTU_MICLU ELONGATION FACTOR TU MICROCOCCUS LUTEUS 221-248

PEFTU_MYCHO ELONGATION FACTOR TU MYCOPLASMA HOMINIS 222-249

PEFTU_MYCLE ELONGATION FACTOR TU ***MYCOBACTERIUM*** LEPRAE 220-257

PEFTU_MYCTU ELONGATION FACTOR TU ***MYCOBACTERIUM*** TUBERCULOSIS 220-247

PEFTU_SHEPU ELONGATION FACTOR TU SHEWANELLA PUTREFACIENS 26-53

PEFTU_STROR ELONGATION FACTOR TU STREPTOCOCCUS ORALIS 232-259

PELAS_PSEAE PSEUDOLYSIN PRECURSOR PSEUDOMONAS AERUGINOSA 141-168

PELT1_ECOLI T-LABILE. . . VIRULENCE PROTEIN PGP7-D CHLAMYDIA TRACHOMATIS
12-60

PGP8D_CHLTR VIRULENCE PROTEIN PGP8-D CHLAMYDIA TRACHOMATIS 94-121

PGREA_RICPR TRANSCRIPTION ELONGA- RICKETTSIA PROWAZEKII 15-49

TION FACTOR GREA

PGRPE_BACSU ***GRPE*** -LIKE PROTEIN BACILLUS SUBTILIS 27-73

PGRPE_BORBU ***GRPE*** -LIKE PROTEIN BORRELIA BURGDORFERI 2-79

PGRPE_CLOAB ***GRPE*** -LIKE PROTEIN CLOSTRIDIUM ACETOBUTYLICUM 12-83

PGRSA_BACBR GRAMICIDIN S SYNTHETASE BACILLUS BREVIS 545-572 799-826 840-882
1035-1062

PGRSB_BACBR GRAMICIDIN S SYNTHETASE BACILLUS BREVIS 48-75 94-121. . .

DETD . . . TYPHIMURIUM 8-35

MEMBRANE Q PROTEIN

PHISX_ECOLI HISTIDINOL DEHYDRO- ESCHERICHIA COLI 393-434

GENASE

PHISX_LACLA HISTIDINOL DEHYDRO- LACTOCOCCUS LACTIS 19-46 264-303

GENASE

PHISX_MYCSM HISTIDINOL DEHYDRO- ***MYCOBACTERIUM*** SMEGMATIS 288-329
399-430

GENASE

PHISX_SALTY HISTIDINOL DEHYDRO- SALMONELLA TYPHIMURIUM 393-434

GENASE

PHLA_STAAU ALPHA-HEMOLYSIN STAPHYLOCOCCUS AUREUS 69-102

PRECURSOR

PHLY1_ECOLI HEMOLYSIN A, CHROMO- ESCHERICHIA COLI. . . SOLANACEARUM 371-405

PHRPH_PSESY OUTER MEMBRANE PROTEIN PSEUDOMONAS SYRINGAE 102-129 310-344

HRPH PRECURSOR

PHRPS_PSESH PROBABLE REGULATORY PSEUDOMONAS SYRINGAE 24-51

PROTEIN HRPS

PHS18_CLOAB 18 KB ***HEAT*** ***SHOCK*** PROTEIN CLOSTRIDIUM
ACETOBUTYLICUM 67-108

PHS70_HALMA ***HEAT*** ***SHOCK*** 70 KD PROTEIN HALOARcula MARISMORTUI
522-576

PHS70_MYCLE ***HEAT*** ***SHOCK*** 70 KD PROTEIN ***MYCOBACTERIUM***
LEPRAE 461-488 503-530

PHS70_MYCPA ***HEAT*** ***SHOCK*** 70 KD PROTEIN ***MYCOBACTERIUM***
PARA- 460-487

TUBERCULOSIS

PHTPG_ECOLI ***HEAT*** ***SHOCK*** PROTEIN C62.5 ESCHERICHIA COLI
221-248 482-509

PHTRA_ECOLI PROTEASE DO PRECURSOR ESCHERICHIA COLI 373-400

PHTRE_ECOLI HTRE PROTEIN PRECURSOR ESCHERICHIA COLI 454-484 524-576

PHTRJ_HALHA SENSORY. . .

DETD . . . 135-162 232-269 288-315

PRECA_METCL RECA PROTEIN METHYLOMONAS CLARA 266-303

PRECA_METFL RECA PROTEIN METHYLOBACILLUS FLAGELLATUM 276-303

PRECA_MYCPU RECA PROTEIN MYCOPLASMA PULMONIS 30-57

PRECA_MYCTU RECA PROTEIN ***MYCOBACTERIUM*** TUBERCULOSIS 749-776

PRECA_NEIGO RECA PROTEIN NEISSERIA GONORRHOEAE 263-310

PRECA_PROMI RECA PROTEIN PROTEUS MIRABILIS 283-310
 PRECA_PSEAE RECA PROTEIN PSEUDOMONAS AERUGINOSA 282-309
 PRECA_RHILP RECA PROTEIN RHIZOBIUM. . . A
 PRPOA_THECE DNA-DIRECTED RNA THERMOCOCCUS CELER 228-262
 POLYMERASE SUBUNIT A'
 PRPOB_ECOLI DNA-DIRECTED RNA ESCHERICHIA COLI 599-626 1011-1038
 POLYMERASE BETA CHAIN
 PRPOB_MYCLE DNA-DIRECTED RNA ***MYCOBACTERIUM*** LEPRAE 723-760 1084-1111
 POLYMERASE BETA CHAIN
 PRPOB_SALTY A-DIRECTED RNA SALMONELLA TYPHIMURIUM 599-626 958-985 1011-1038
 POLYMERASE BETA CHAIN
 PRPOB_SULAC A-DIRECTED RNA SULFOLOBUS ACIDOCALDARIUS. . . HALOCOCCUS
 MORRHUAE 27-54 117-144 207-234
 POLYMERASE SUBUNIT C
 PRPOC_METTH DNA-DIRECTED RNA METHANOBACTERIUM 58-85 272-302 327-354
 POLYMERASE SUBUNIT C THERMOAUTOTROPHICU
 PRPOC_MYCLE A-DIRECTED RNA ***MYCOBACTERIUM*** LEPRAE 273-300 860-887
 911-938 1131-1158
 POLYMERASE BETA' CHAIN
 PRPOC_NOSCO DNA-DIRECTED RNA NOSTOC COMMUNE 150-192
 POLYMERASE GAMMA CHAIN
 PRPOC_SULAC DNA-DIRECTED RNA SULFOLOBUS ACIDOCALDARIUS. . . 35-62 182-216
 PRS6_THETH 30S RIBOSOMAL PROTEIN S6 THERMUS AQUATICUS 16-43
 PRS7_METVA 30S RIBOSOMAL PROTEIN S7 METHANOCOCCUS VANNIELII 69-96
 PRS7_MYCLE 30S RIBOSOMAL PROTEIN S7 ***MYCOBACTERIUM*** LEPRAE 22-49
 PRS8_MICLU 30S RIBOSOMAL PROTEIN S8 MICROCOCCUS LUTEUS 103-130
 PRS8_MYCCA 30S RIBOSOMAL PROTEIN S8 MYCOPLASMA CAPRICOLUM 41-78
 PRSGA_ECOLI FERRITIN LIKE PROTEIN ESCHERICHIA. . .
 DETD . . . COLI 181-208 308-340 720-754
 PTRAC6_ECOLI TRANSPOSASE ESCHERICHIA COLI 51-78
 PTRAC6_SHISO TRANSPOSASE SHIGELLA SONNEI 51-78 200-227 231-258
 PTRAC7_ECOLI TRANSPOSASE ESCHERICHIA COLI 729-756
 PTRAC9_MYCTU PUTATIVE TRANSPOSASE ***MYCOBACTERIUM*** TUBERCULOSIS 159-186
 PTRAB_BACTB IS231B PROBABLE BACILLUS THURINGINENSIS 281-308 419-446
 TRANSPOSASE
 PTRAC_BACTB IS231C PROBABLE BACILLUS THURINGIENSIS 281-308 419-446
 TRANSPOSASE
 PTRAC_STAAU TRANSPOSASE STAPHYLOCOCCUS AUREUS 4-31. . .
 DETD . . . HYPOTHETICAL PROTEIN ESCHERICHIA FERGUSONII 2-35
 PYAM1_SALTY PUTATIVE AMIDASE SALMONELLA TYPHIMURIUM 73-100
 PYAT1_SYNY3 HYPOTHETICAL 13.0 KD SYNECHOCYSTIS SP 26-60
 PROTEIN
 PYATP_MYCLE HYPO PROTEIN PUTATIVE ***MYCOBACTERIUM*** LEPRAE 23-57 91-158
 511-538
 ATP OPERON
 PYATR_BACFI HYPOL ATP-BINDING BACILLUS FIRMUS 211-238
 TRANSPORT PROTEIN
 PYATS_MYCGA HYPOTHETICAL PROTEIN MYCOPLASMA GALLISEPTICUM 7-41
 PYATU_MYCGA HYPOTHETICAL PROTEIN. . . LACTOBACILLUS HELVETICUS 93-120
 127-154
 PYHYA_PSESN HYPOTHETICAL PROTEIN PSEUDOMONAS SP 217-266
 PYI11_HALHA HYPOTHETICAL 38.0 KD HALOBACTERIUM HALOBIUM 245-272
 PROTEIN
 PYI32_MYCTU IS986 HYPOTHETICAL 6.6 KD ***MYCOBACTERIUM*** TUBERCULOSIS
 19-46
 PROTEIN
 PYI42_PSEAY HYPOTHETICAL 42.6 KD PSEUDOMONAS AMYLODERAMOSA 9-36

PROTEIN
PYI48_METSM ISM1 HYPOTHETICAL 48.3 KD METHANOBREVIBACTER SMITHII 73-100
154-184 338-365

PROTEIN

PYI52_HALHA. . .

DETD . . . GROWTH FACTOR PRECURSOR, KIDNEY (EGF) 47-74

(UROGASTRONE)

PELF1_HUMAN ETS-RELATED TRANSCRIPTION FACTOR ELF-1 551-588

PENPL_HUMAN ENDOPLASMIN PRECURSOR (94 KD GLUCOSE-REGULATED 47-74 246-273
PROTEIN) (***GRP94***) (GP96

PENV1_HUMAN RETROVIRUS-RELATED ENV POLYPROTEIN 382-420

PEPC_HUMAN IG EPSILON CHAIN C REGION 161-188

PEPMO_HUMAN EPIMORPHIN 35-62 67-94 249-283

PER72_HUMAN PROTEIN DISULFIDE ISOMERASE-RELATED PRECURSOR 58-85. . .

DETD . . . HYDROXYMETHYLTRANSFERASE, CYTOSOLIC 32-59 344--371

(EC 2.1.2.1) (SERINE

PGLY2_HUMAN SERINE HYDROXYMETHYLTRANSFERASE, MITOCHONDRIAL 417-444

(EC 2.1.2.1) (SERINE

PGR78_HUMAN 78 KD GLUCOSE REGULATED PROTEIN PRECURSOR (***GRP*** 78)
564-591 598-625

(IMMUNOGLOBULIN

PGRA2_HUMAN GLYCINE RECEPTOR ALPHA-2 CHAIN PRECURSOR 142-169 341-368

PGRAV_HUMAN GRAVIN (FRAGMENT) 9-43 61-88

PGRFR_HUMAN GROWTH HORMONE-RELEASING HORMONE RECEPTOR 128-155

. . . 1.13.11.27) 306-333

(4HPPD)

PHRX_HUMAN ZINC FINGER PROTEIN HRX 521-548 914-974 1637-1666 2215-2286

2289-2316 3317-3344 3448-3475

PHS1_HUMAN HEMATOPOIETIC LINEAGE CELL SPECIFIC PROTEIN 43-70

PHS9A_HUMAN ***HEAT*** ***SHOCK*** PROTEIN HSP 90-ALPHA (HSP 86)
443-470 640-674

PHSER_HUMAN HEAT-STABLE ENTEROTOXIN RECEPTOR PRECURSOR (GC-C) 511-545

(INTESTINAL

PHSF1_HUMAN ***HEAT*** ***SHOCK*** FACTOR PROTEIN 1 (HSF 1) (
HEAT ***SHOCK*** 113-140 168-209

TRANSCRIPTION FACTOR

PHSF2_HUMAN ***HEAT*** ***SHOCK*** FACTOR PROTEIN 2 (HSF 2) (
HEAT ***SHOCK*** 117-198

TRANSCRIPTION FACTOR

PHV2I_HUMAN IG HEAVY CHAIN PRECURSOR V-II REGION (ARH-77) 67-108

PHV3T_HUMAN IG HEAVY CHAIN V-III REGION (GAL) 47-74

PHX11_HUMAN HOMEBOX PROTEIN. . .

DETD . . . Leu Asp Lys Tyr

20

25

30

Lys Asn Ala

35

SEQUENCE CHARACTERISTICS:

LENGTH: 35 amino acids

TYPE: amino acid

STRANDEDNESS:

TOPOLOGY: unknown

MOLECULE TYPE: peptide

FEATURE:

NAME/KEY: ***Modified*** -site

OTHER INFORMATION: / ***product*** = "OTHER"
represents U, the standard designation
modified cysteine."

/note= "X
for C-abu, a

SEQUENCE: 127

Ser Asn Ile Lys Glu Asn Lys. . . Val Thr Glu Leu
20 25 30

Gln Leu Leu

35

SEQUENCE CHARACTERISTICS:

LENGTH: 35 amino acids

TYPE: amino acid

STRANDEDNESS:

TOPOLOGY: unknown

MOLECULE TYPE: peptide

FEATURE:

NAME/KEY: ***Modified*** -site

OTHER INFORMATION: / ***product*** = "OTHER"

represents U, the standard designation

modified cysteine."

/note= "X
for C-abu, a

SEQUENCE: 128

Lys Glu Asn Lys Xaa Asn Gly. . .

L13 ANSWER 7 OF 9 USPATFULL on STN

AN 2002:315069 USPATFULL

TI Compositions and methods for treatment of neoplastic disease

IN Terman, David S., Pebble Beach, CA, UNITED STATES

PI US 2002177551 A1 20021128

AI US 2001-870759 A1 20010530 (9)

PRAI US 2000-208128P 20000531 (60)

DT Utility

FS APPLICATION

LREP David S. Terman, P.O. Box 987, Pebble Beach, CA, 93953

CLMN Number of Claims: 30

ECL Exemplary Claim: 1

DRWN 3 Drawing Page(s)

LN.CNT 17323

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention comprises compositions and methods for treating a tumor or neoplastic disease in a host, The methods employ conjugates comprising superantigen polypeptides, nucleic acids with other structures that preferentially bind to tumor cells and are capable of inducing apoptosis. Also provided are superantigen-glycolipid conjugates and vesicles that are loaded onto antigen presenting cells to activate both T cells and NKT cells. Cell-based vaccines comprise tumor cells engineered to express a superantigen along with glycolipids products which, when expressed, render the cells capable of eliciting an effective anti-tumor immune response in a mammal into which these cells are introduced. Included among these compositions are tumor cells, hybrid cells of tumor cells and accessory cells, preferably dendritic cells. Also provided are tumoricidal T cells and NKT cells devoid of inhibitory receptors or inhibitory signaling motifs which are hyperresponsive to the the above compositions and lipid-based tumor associated antigens that can be administered for adoptive immunotherapy of cancer and infectious diseases.

SUMM . . . cell both enterotoxins and .alpha.-galactosylceramides are associated with numerous intracellular and membrane structures such as MHC, costimulatory and adhesion molecules, ***heat*** ***shock*** proteins, membrane glycolipids and glycosphingolipids which may improve immunogenicity and antigen presentation. They may also be transported in various vesicles. . . .

SUMM . . . structures may actually improve the T cell activating function of SAGs such as deoxyribonucleic acids, ribonucleic acids, tumor associated antigens, ***heat*** ***shock*** proteins, costimulatory molecules and adhesion molecules and endosomes. Cellular SAG peptides or nucleotides exist in association with tumor associated antigens, costimulants, adhesion molecules, ***heat*** ***shock*** proteins and MHC molecules, GPI-ceramides or SAG receptors (digalactosylceramides) which improve the immunogenicity of the tumor antigens. Therefore, these structural. . .

SUMM . . . tumor cells, an immunogenic bacterial product such as Staphylococcal adhesin protein A, LPS, .beta.-glucans, and peptidoglycans, costimulatory and adhesion molecules, ***heat*** ***shock*** protein, growth factor receptors such as Her/neu and tumor markers such as PSA.

DRWD . . . Oncogenes, amplified oncogenes and transcription factors

15. Angiogenic factors and receptors

16. Tumor growth factor receptors

17. Tumor suppressor receptors

18. Cell cycle proteins

19. ***Heat*** - ***shock*** proteins, ATPases and G proteins

20. Proteins engaged in antigen processing, sorting and intracellular trafficking

21. Inducible nitric oxide synthase (iNOS)

22. apolipoproteins. . .

DETD . . . the ability to stimulate large subsets of T cells. SAGs include Staphylococcal enterotoxins, Streptococcal pyrogenic exotoxins, mycoplasma antigens, rabies antigens, ***mycobacteria*** antigens, EB viral antigens, minor lymphocyte stimulating antigen, mammary tumor virus antigen, ***heat*** ***shock*** proteins, stress peptides, clostridial and toxoplasmosis antigens and the like. Any SAG can be used as described herein, although, Staphylococcal. . .

DETD . . . in gram positive bacteria (such as Staphylococcus or Streptococcus), to nucleic acids encoding capsular polysaccharides and teichoic acids and in ***mycobacterial*** species to nucleic acids encoding lipoarabinan.

DETD . . . E. coli, Salmonella or Klebsiella or for bacteria which naturally produce lipoarabinomannans glycans or polysaccharides containing cell walls such as ***mycobacterium*** and streptococcus respectively. The SAG-polysaccharide constructs bind to CD1 receptors of antigen presenting cells. They are then capable of activating. . .

DETD . . . J et al., J. Natl. Cancer Inst. 72:955-962 (1984)). By synthesizing single stranded nucleotides corresponding to different regions in the ***Mycobacterium*** bovis genome, specific single stranded oligonucleotides that activate adherent splenocytes and enhanced natural killer cell activity have been identified. In. . .

DETD [0218] Examples are LPS's of gram negative organisms, SAGs and peptidoglycans of gram positive organisms, fungal .beta.-glucans, bacterial glycosylceramides, and ***mycobacterial*** lipoarabinans. Numerous infectious agents with these structures cause potent immune reactions e.g. streptococcal cellulitis induced by S. pyogenes, E. coli. . .

DETD . . . molecule interact with different types of host cells. There is also evidence that immunopotentiating activity of a glycopeptide produced by ***mycobacteria*** is dependent on the saccharide residues of the molecule.

DETD [0249] Genes Involved in ***Mycobacterial*** Cell Wall Biosynthesis

DETD . . . fused in frame or cotransfected into tumor cell with nucleic acids encoding the key enzymes involved in the biosynthesis of ***mycobacterial*** cell wall mycolic acid, phosphatidylinositol mannosides and lipoarabinans. A high affinity interaction of CD1b molecules with the acyl side chains. . . .

DETD . . . in the biosynthesis of these molecules have been isolated. In addition to the usual fatty acids found in membrane lipids, ***mycobacteria*** have a wide variety of very long-chain saturated (C18-C32) and monounsaturated (up to C26) n-fatty acids. The occurrence of a-alkyl b-hydroxy very long chain fatty acids i.e., mycolic acid is a hallmark of ***mycobacteria*** and related species. ***Mycobacterial*** mycolic acids are the largest (C70-C90) with the largest -branch (C20-C25). The main chain contains one or two double bonds,. . . esterified to glycerol or sugars such as trehalose, glucose and fructose depending on the sugars present in the culture medium. ***Mycobacterium*** also contains several methyl-branched fatty acids. These include 10-methyl C18 fatty acid (tuberculostearic acid found esterified in phosphatidyl inositide mannosides),. . .

DETD [0255] The MAS gene encoding ***mycobacterial*** mycocerosic acid synthase is a dimer of the FAS gene. The cloning and sequencing of the MAS gene revealed the. . .

DETD . . . results in tissue specific forms of the protein, which can be intracellular, membrane bound, or secreted. In cells infected with ***mycobacteria***, the CD1 molecule binds and presents a ***mycobacterial*** membrane component, mycolic acid. Surface CD1 molecules present longer peptides than those normally found on class I molecules. Whether CD1. . .

DETD . . . Nucleic acid encoding cell wall or cell membrane associated glycosylceramides or a branched, b hydroxy long-chain fatty acids found in ***mycobacteria*** and other bacteria are cotransfected into the CD1 transfected tumor cells. The tumor cell therefore displays glycosylceramides bound to the. . .

DETD [0366] 36. SAGs Combined with Signal Transduction Molecules or ***Heat*** ***Shock*** Proteins (HSPs)

DETD . . . frame to (or cotransfected with) a nucleic acid encoding "signal transduction molecules" such as Ras, JAK 1 and STAT-1a and ***heat*** ***shock*** proteins HSP-60, HSP-70, HSP-90a, HSP-90b, Cox-2 as well as heterotrimeric G proteins and ATPases. The genes for Staphylococcal HSP-70 (SEQ. . .

DETD . . . not limited to two recently discovered HSP genes, orf37 and orf35 in Staphylococcus aureus that are upstream and downstream of ***grpE*** (hsp20), dnaK (hsp70) and dnaJ (hsp40) in the following sequence: orf37-hsp20-hsp70-hsp40-orf35. The promoters are located upstream of orf37 and upstream. . .

DETD . . . pathway. Owing to the lower B-carotene content, Lp(a) may be more easily oxidized than LDL. Oxidized Lp(a) such as Lp(a) ***modified*** by malondialdehyde, a ***product*** generated in vivo from aggregated platelets, is avidly taken up by monocyte-macrophages, through the scavenger-receptor pathway. Lp(a) accumulates in either. . .

DETD [0589] IR.sub.IDAs recognize Lip-IDAs derived from bacteria, ***mycobacteria***, parasites, fungi, protozoans or plants and respond by producing an inhibitory T cell response. Lip-IDAs comprise sphingolipids, glycopeptides, phytoglycolipids, mycoglycolipids,. . .

DETD . . . types listed above). In another embodiment, SAGs are conjugated to Lip-IDAs such as glycans and peptidoglycan antigens derived from

bacteria, ***mycobacteria***, parasites, fungi or plants. These families are listed above. These lipid based molecules also include sphingolipids with inositolphosphate-containing head groups. . .

DETD [0614] Conjugates between SAg and a Lip-IDA derived from a fungal, parasitic or ***mycobacterial*** sources are also used for the treatment of infectious diseases such as tuberculosis, leishmaniasis, trypanosomiasis as disclosed in Example 53.. . . are also useful ex vivo for activating a population of cells in which IR.sub.IDAs specific for bacterial, fungal, parasitic or ***mycobacterial*** antigens have been (1) deleted (via gene knockout) or (2) functionally inactivated (via antisense) for use in adoptive immunotherapy of. . .

DETD . . . 92: 1619-1623
(1995)

31. Lipid A biosynthetic (SEQ ID NOS:105-112) Tumor
Schnaitman CA et al.,
genes lpxA-D
Microbiological
Reviews 57: 655-682
(1993)

32. ***Mycobacterial*** mycolic acid (SEQ ID NOS:113-114) Tumor
Fernandes ND et al.,
Gene 170: 95-99 (1996);
Mathur M et al., J.Biol.
Chem.. . .

DETD . . . and greatly restricted anchors are preferred. This recognition of CD1-presented antigens depends on the type and distribution of sugar residues. ***Mycobacterial*** cell wall antigens namely mycolic acids and lipoarabinomannan also bind to CD1. Recently several glycosylceramides, in particular, monogalactosyl ceramides GalCer). . .

DETD . . . or exosomes comprising SAg-GalCer complexes or SAg-tumor peptide (including but not limited to normal mutated structures). The ternary complexes of SAg-GalCer- ***heat*** ***shock*** protein and tumor peptide- ***heat*** ***shock*** protein are also useful, These complexes may be in or soluble or immobilized form, attached to a CD1 or MHC. . .

DETD . . . acid and growth factor receptor nucleic acids18. SAg-encoding nucleic acid and cell cycle protein nucleic acids

19. SAg-encoding nucleic acid and ***heat*** ***shock*** protein nucleic acids

20. SAg-encoding nucleic acid and chemokine nucleic acids

21. SAg-encoding nucleic acid and cytokine nucleic acids

22. SAg-encoding nucleic acid. . .

DETD . . . molecules containing amino acid sequences and homologous to the enterotoxin family of molecules. To this extent, mammary tumor virus sequences, ***heat*** ***shock*** proteins, stress peptides, Mycoplasma and ***mycobacterial*** antigens, and minor lymphocyte stimulating loci bearing tumoricidal structural homology to the enterotoxin family are useful as anti-tumor agents. Hybrid. . .

DETD . . . commonly used inducible promoters include the metallothionein CUP1 promoter, which is tightly controlled by copper; promoters activated in response to ***heat*** ***shock***, which are of particular interest for expression in the temperature-sensitive sec6-4 mutant and the PH05 promoter, which is derepressed at. . .

DETD . . . you using beige mutants on a B6 background? This is not clear here are infected with many of the nontuberculous ***mycobacteria*** : MAC (what is this), M. kansasii, M. simiac, M. malmoense or M.

genavense. Same-sex mice aged 5-7 weeks are allowed. . .
DETD [1853] Primary cultures of MAC (M. kansasii or other
mycobacteria) to be used for infection are obtained from
clinical isolates of patients with disseminated MAC infection, or the
American Type. . .
DETD . . . bacilli in the lungs the infection grows progressively at first
and is then curtailed around 20 days. Laboratory strains of
mycobacteria such as Erdman attain 4-5 logs in the lungs by
this
time. More virulent strains such as CSU93 (Tennessee outbreak). . .
DETD [1918] Preparation of Lip-TAAs and Lip-IDAs of Bacterial, Fungal, Yeast,
Parasitic, ***Mycobacterial*** , Invertebrate or Protozoan Origin

L13 ANSWER 8 OF 9 USPATFULL on STN

AN 2002:307566 USPATFULL

TI Methods and compositions for therapeutic intervention in infectious
disease

IN Stewart, Graham, Walton-on-Thames, UNITED KINGDOM

O'Gaora, Peadar, London, UNITED KINGDOM

Young, Douglas, Ruislip, UNITED KINGDOM

PI US 2002172685 A1 20021121

AI US 2002-79136 A1 20020220 (10)

PRAI US 2001-269801P 20010220 (60)

US 2001-294170P 20010529 (60)

DT Utility

FS APPLICATION

LREP JOHN S. PRATT, ESQ, KILPATRICK STOCKTON, LLP, 1100 PEACHTREE STREET,
SUITE 2800, ATLANTA, GA, 30309

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 15 Drawing Page(s)

LN.CNT 1922

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods and compositions for the treatment and prevention of infectious
diseases are provided. In particular, efficient vaccines comprising
genetically modified pathogens are provided. The vaccines generally
comprise ***mycobacterial*** mutants having ***modified***
protein ***production*** capabilities. In one embodiment, the
mutants overexpress ***heat*** ***shock*** protein. In a
specific embodiment, the ***mycobacterial*** mutant overexpresses
heat ***shock*** proteins 60 and/or 70. Also provided are
modified BCG vaccines capable of overexpressing ***heat***
shock proteins 60 and/or 70.

AB . . . prevention of infectious diseases are provided. In particular,
efficient vaccines comprising genetically modified pathogens are
provided. The vaccines generally comprise ***mycobacterial***
mutants having ***modified*** protein ***production***
capabilities. In one embodiment, the mutants overexpress ***heat***
shock protein. In a specific embodiment, the
mycobacterial mutant overexpresses ***heat*** ***shock***
proteins 60 and/or 70. Also provided are modified BCG vaccines capable
of overexpressing ***heat*** ***shock*** proteins 60 and/or 70.

SUMM . . . the invention relates to the manipulation of antigen production
by infectious organisms. More particularly, the present invention
comprises manipulation of ***mycobacterial*** genes resulting in the
modification of ***heat*** ***shock*** protein production.

SUMM [0003] ***Mycobacterial*** infections often manifest as diseases

such as tuberculosis. Human infections caused by ***mycobacteria*** have been widespread since ancient times, and tuberculosis remains a leading cause of death today. Although the incidence of the disease declined in parallel with advancing standards of living since at least the mid-nineteenth century, ***mycobacterial*** diseases still constitute a leading cause of morbidity and mortality in countries with limited medical resources and can cause overwhelming, disseminated disease in immunocompromised patients. In spite of the efforts of numerous health organizations worldwide, the eradication of

mycobacterial diseases has never been achieved, nor is eradication imminent. Nearly one third of the world's population is infected with M. . . .

SUMM [0005] Approximately half of all patients with acquired immune deficiency syndrome (AIDS) will acquire a ***mycobacterial*** infection, with TB being an especially devastating complication. AIDS patients are at higher risks of developing clinical TB and anti-TB. .

SUMM [0006] ***Mycobacteria*** other than M. tuberculosis are increasingly found in opportunistic infections that plague the AIDS patient. Organisms from the M. avium-intracellulare complex (MAC), especially serotypes four and eight, account for 68% of the ***mycobacterial*** isolates from AIDS patients. Enormous numbers of MAC are found (up to 10^{sup}.10 acid-fast bacilli per gram of tissue) and, . . .

SUMM [0007] Crohn's disease is a chronic inflammatory bowel disease characterized by transmural inflammation and granuloma formation. ***Mycobacterium*** avium subspecies paratuberculosis (M. paratuberculosis) causes a similar disease in animals. Johnes's disease, affecting cattle, causes estimated losses of \$1.5. . . .

SUMM [0008] Cattle also suffer from infection with ***Mycobacterium*** bovis which causes a disease similar to tuberculosis. Control of infection is a serious herd management concern. This infection can. .

SUMM . . . of new therapeutic agents that are effective as vaccines and as treatments for disease caused by drug resistant strains of

mycobacteria .
SUMM [0011] Although over 37 species of ***mycobacteria*** have been identified, more than 95% of all human infections are caused by six species of ***mycobacteria*** : M tuberculosis, M. avium-intracellulare, M. kansasii, M. fortuitum, M. chelonae, and M. leprae. The most prevalent ***mycobacterial*** disease in humans is tuberculosis (TB) which is caused by ***mycobacterial*** species comprising M. tuberculosis, M. bovis, or M. africanum (Merck Manual, 1992). Infection is typically initiated by the inhalation of. . .

SUMM [0012] There is still no clear understanding of the factors which contribute to the virulence of ***mycobacteria*** . Many investigators have implicated lipids of the cell wall and bacterial surface as contributors to colony morphology and virulence. Evidence suggests that C-mycosides, on the surface of certain ***mycobacterial*** cells, are important in facilitating survival of the organism within macrophages. Trehalose 6,6' dimycolate, a cord factor, has been implicated for other ***mycobacteria*** .

SUMM [0014] Diagnosis of ***mycobacterial*** infection is confirmed by the isolation and identification of the pathogen, although conventional diagnosis is based on sputum smears, chest X-ray examination (CXR), and clinical symptoms. Isolation of ***mycobacteria*** on a medium takes as long a time as four to eight weeks. Species identification takes a

further two weeks. There are several other techniques for detecting
 mycobacteria such as the polymerase chain reaction (PCR),
 mycobacterium tuberculosis direct test, or amplified
 mycobacterium tuberculosis direct test (MTD), and detection
 assays that utilize radioactive labels.

SUMM . . . and many times, the results are inaccurate as false positives
 are sometimes seen in subjects who have been exposed to
 mycobacteria but are healthy. In addition, instances of
 mis-diagnosis are frequent since a positive result is not observed only
 in active TB patients, but also in BCG-vaccinated persons and those who
 had been infected with ***mycobacteria*** but have not developed the
 disease. It is hard therefore, to distinguish active TB patients from
 the others, such as. . . by the tuberculin skin test. Additionally,
 the tuberculin test often produces a cross-reaction in those individuals
 who were infected with ***mycobacteria*** other than M tuberculosis
 (MOTT). Diagnosis using the skin tests currently available is frequently
 subject to error and inaccuracies.

SUMM . . . no longer consistently effective as a result of the problems
 with treatment compliance contributing to the development of drug
 resistant ***mycobacterial*** strains.

SUMM . . . infectious organism genes resulting in the modification of
 protein production are provided. Specifically, the present invention
 provides a teaching of ***mycobacterial*** genetic manipulation
 which results in an increase in ***heat*** ***shock*** protein
 production. The increase in ***heat*** ***shock*** protein
 production results in an enhanced immune response to the ***heat***
 shock proteins and also other ***mycobacterial*** proteins
 in general.

SUMM [0021] ***Heat*** ***shock*** proteins (hsp) are widely
 distributed in nature and are among the most highly conserved molecules
 of the biosphere. ***Heat*** ***shock*** proteins perform
 important functions in the folding and unfolding or translocation of
 proteins, as well as in the assembly and disassembly of protein
 complexes. Because of these helper functions, ***heat***
 shock proteins have been termed molecular chaperones.
 Heat ***shock*** protein synthesis is increased to protect
 prokaryotic or eukaryotic cells from various insults during periods of
 stress caused by infection, . . .

SUMM . . . inventors of the present invention provide for the first time a
 teaching of the use of pathogenic, and more specifically
 mycobacterial , ***heat*** ***shock*** proteins in novel
 vaccines and therapeutics. The findings of the inventors are both
 unobvious and unexpected since those skilled in the art have not
 considered the use of ***heat*** ***shock*** proteins in this
 capacity. For example, Zugel et al. state that "although hsp play an
 important role in several infectious and autoimmune diseases, evidence
 arguing against the direct involvement of ***heat*** ***shock***
 proteins in protection or autoaggression has been gathered. At present,
 initiation of protective immunity against infectious antigens or
 autoimmune disorders by ***heat*** ***shock*** proteins alone
 appears unlikely." (Zugel et al. Clinical Microbiology Reviews 12(1) pp
 19-39 (1999) (emphasis added)).

SUMM [0024] The vaccination methods described herein involve the manipulation
 of ***mycobacterial*** protein production. Such proteins include,
 but are not limited to, ***mycobacterial*** ***heat***
 shock proteins such as ***heat*** ***shock*** protein

(Hsp60) (GroEL1, Rv3417c:GroEL2, Rv0440), Hsp10 (GroES, Rv3418c), Hsp70 (Rv0350), DnaJ (Hsp40, Rv0352), ***GrpE*** (Rv0351) and ***ClpB*** (Rv0384c) and Hsp90. A particularly preferred embodiment of the invention comprises a mutant strain of *M. tuberculosis* that constitutively overexpresses Hsp70. Another preferred embodiment of the present invention comprises *M. bovis* BCG (hereafter "BCG") vaccines capable of ***heat*** ***shock*** protein overexpression. In another preferred embodiment, mutant strains of ***mycobacteria*** or BCG overexpress more than one ***heat*** ***shock*** protein; such mutants include for example, strains that overexpress both Hsp70 and Hsp60. The present invention contemplates other combinations of ***heat*** ***shock*** protein overexpression. The present invention further contemplates overexpression of other ***mycobacterial*** proteins such as antigenic proteins found in the cell wall or secreted by the pathogen.

SUMM [0026] Another object of the present invention is to provide methods and compositions for the treatment and prevention of ***mycobacterial*** disease such as tuberculosis.

SUMM [0027] It is another object of the present invention to provide methods and compositions for the treatment and prevention of ***mycobacterial*** disease using compositions comprising genetically altered ***mycobacteria*** that are capable of overexpressing certain proteins.

SUMM . . . present invention is to provide methods and compositions for the treatment and prevention of tuberculosis using compositions comprising genetically altered ***mycobacteria*** that overexpress certain proteins, wherein the proteins comprise ***heat*** ***shock*** proteins, cell wall proteins or other antigenic proteins secreted by the pathogen.

SUMM . . . to provide methods and compositions for the treatment and prevention of tuberculosis wherein the proteins overexpressed by the genetically altered ***mycobacteria*** comprise Hsp60, Hsp70 and various combinations thereof.

SUMM [0030] Another object of the present invention is to provide compositions for vaccine formulations for the prevention of ***mycobacterial*** disease.

SUMM [0032] Yet another object of the present invention is to provide compositions for vaccine formulations for the prevention of ***mycobacterial*** disease caused by ***mycobacterial*** species comprising *M. tuberculosis* complex, *M. avium-intracellulare*, *M. kansasii*, *M. fortuitum*, *M. chelonae*, *M. leprae*, *M. africanum*, and *M. microti*.

SUMM [0033] Another object of the present invention is to provide methods for the manipulation of pathogenic organisms, namely ***mycobacterial*** genes, resulting in the modification of protein production.

SUMM [0034] It is yet another object of the present invention to provide methods and compositions for production of ***mycobacterial*** mutants characterized by a defective ***heat*** ***shock*** response.

SUMM [0035] Another object of the present invention is to provide methods and compositions for production of ***mycobacterial*** mutants wherein the hspR gene of *M. tuberculosis* has been modified resulting in the overexpression of Hsp70.

SUMM [0036] Another object of the present invention is to provide methods and compositions for production of ***mycobacterial*** mutants wherein the hspR gene of BCG has been modified resulting in the overexpression of Hsp70.

SUMM [0037] Another object of the present invention is to provide methods and compositions for production of ***mycobacterial*** mutants wherein the hrcA gene of M. tuberculosis has been modified resulting in the overexpression of Hsp60.

SUMM [0038] It is another object of the present invention to provide methods and compositions for production of ***mycobacterial*** mutants wherein the hrcA gene of M. bovis has been modified resulting in the overexpression of Hsp60.

SUMM [0039] Yet another object of the present invention is to provide methods and compositions for production of ***mycobacterial*** mutants wherein both the hspR and hrcA genes of M. tuberculosis have been modified resulting in the overexpression of both. . . .

SUMM [0040] Another object of the present invention is to provide methods and compositions for production of ***mycobacterial*** mutants wherein both the hspR and hrcA genes of BCG have been modified resulting in the overexpression of both Hsp70,

DRWD [0046] c. Southern blot analysis of PstI digested genomic DNA probed with the HS1/HS2 PCR product corresponding to ***grpE*** and dnaJ. Lane 1, .lambda. HindIII ladder; lane 2, M. tuberculosis H37Rv; lane 3, M tuberculosis hspR mutant.

DRWD points for the hsp70 operon using mRNA extracted from wild type BCG (WT) and the .DELTA.hspR mutant with and without ***heat*** ***shock*** .

DRWD [0049] b. SDS-PAGE analysis of [.sup.35S]-methionine-labeled proteins from wild type BCG (WT) and the .DELTA.hspR mutant with and without ***heat*** ***shock*** .

DRWD [0070] FIG. 9. SDS-PAGE showing overexpressed ***ClpB*** , Hsp70, Hsp60 and Hsp10 (GroES) in the hspR and hrcA deleted strain. Lane 1, wild type M. tuberculosis H37Rv; lane. . . .

DRWD [0071] FIG. 10. Gene expression profiles of M. tuberculosis during ***heat*** ***shock*** and of M. tuberculosis lacking the transcriptional repressor, HspR. Scatter plots show log Cy5/Cy3 signal ratios against log total signal. . . .

DRWD [0072] FIG. 11. Functional distribution of genes upregulated during ***heat*** ***shock*** . Frequency of genes among functional groups

(<http://genolist.pasteur.fr/TubercuList/>) across the genome (grey bars) and among ***heat*** ***shock*** induced genes (black bars).

DRWD [0073] FIG. 12. ***Heat*** ***shock*** repressor binding sites within M. tuberculosis. A, HspR associated inverted repeat or HAIR sequences. B, HrcA binding sites or CIRCE. . . .

DETD [0079] ***Mycobacterial*** infections such as those causing tuberculosis, once thought to be declining in occurrence, have rebounded and again constitute a serious. . . . threat. Areas where humans are crowded together or living in substandard housing are increasingly found to have persons infected with ***mycobacteria*** . Persons who are immunocompromised are at great risk of being infected with ***mycobacteria*** and dying from such infection. In addition, the emergence of drug-resistant strains of ***mycobacteria*** has added to the treatment problems of such infected persons.

DETD [0080] Many people who are infected with ***mycobacteria*** are poor or live in areas with inadequate health care facilities. As a result of various obstacles (economical, education levels. . . . these and other individuals results in the prevalence of disease frequently compounded by the emergence of drug resistant strains of ***mycobacteria*** . Effective vaccines that target various strains of ***mycobacteria*** are necessary to bring the increasing numbers of tuberculosis under

control.

DETD [0081] The present invention provides methods and compositions comprising genetically modified pathogenic organisms such as ***mycobacteria*** for the prevention and treatment of infectious disease such as tuberculosis. More particularly, the present invention provides ***mycobacterial*** mutants capable of altered protein expression. As described herein, the protein that has altered expression may be overexpressed and may comprise any relevant ***mycobacterial*** protein, such as a cell wall protein or other antigenic protein secreted by the pathogen. Typically, the overexpressed protein is a ***heat*** ***shock*** protein such as Hsp60 or Hsp70. In an alternative embodiment of the present invention, `multiple` mutants i.e. genetically modified ***mycobacteria*** capable of altered expression of more than one protein, are also provided. In a particular embodiment, `double` mutants capable of. . .

DETD [0082] In addition to the above-described embodiments, the present invention also provides improved BCG vaccines capable of overexpressing ***heat*** ***shock*** proteins. In a most preferred embodiment, a

vaccine comprising BCG capable of overexpressing both Hsp60 and Hsp 70 and co-regulated. . .

DETD [0083] The methods and compositions of the present invention may be used for vaccinating and treating ***mycobacteria*** infection in humans as well as other animals. For example, the present invention may be particularly useful for the prevention. . .

DETD [0084] As used herein the term "tuberculosis" comprises disease states usually associated with infections caused by ***mycobacteria*** species comprising M. tuberculosis complex. ***Mycobacterial*** infections caused by ***mycobacteria*** other than M. tuberculosis (MOTT) are usually caused by ***mycobacterial*** species comprising M. avium-intracellulare, M. kansasii, M. fortuitum, M. chelonae, M. leprae, M. africanum, M. microti and M. paratuberculosis.

DETD [0085] Elevated expression of ***heat*** ***shock*** proteins can benefit a microbial pathogen struggling to penetrate host defenses during infection, but at the same time may provide. . . its ability to persist during the subsequent chronic phase. As demonstrated herein, the present inventors discovered that induction of microbial ***heat*** ***shock*** genes provides a novel strategy to boost the immune response of individuals harboring latent tuberculosis infection.

DETD [0086] Cells exposed to elevated temperature or other stress stimuli respond by increased expression of ***heat*** ***shock*** proteins..sup.1 The ***heat*** ***shock*** response, and the proteins involved, have been highly conserved throughout evolution from Escherichia coli to man. The major ***heat*** ***shock*** proteins are molecular chaperones with an essential role in directing folding and assembly of polypeptides within the cell..sup.2 Enhanced expression of ***heat*** ***shock*** proteins in response to stress allows cells to tolerate potentially harmful consequences associated with intracellular accumulation of denatured polypeptides.

DETD [0087] Synthesis of ***heat*** ***shock*** proteins is induced in microbial pathogens during infection.sup.3-5. While the increased level of these proteins is likely to enhance microbial. . . have discovered that it may also provide an important signal in alerting the host to the presence of the pathogen. ***Heat*** ***shock*** proteins interact with the immune system through a variety of mechanisms. They were initially identified as prominent antigens in a.

. . . as chaperones is associated with an ability to promote immune responses to other polypeptides.sup.8,9. Finally, although the functional role of ***heat*** ***shock*** proteins is primarily intracellular, several studies suggest that exogenous ***heat*** ***shock*** proteins trigger immunomodulatory signals as a result of recognition by cell surface receptors.sup.10-12.

DETD [0088] Current knowledge in this area provides that ***heat*** ***shock*** proteins are mainly associated with disease and that these proteins are "virulence factors" that constitute the part of the ***mycobacterial*** organism that is fundamentally responsible for disease. Contrary to current knowledge however, the present inventors have examined the role of. . . more than wildtype and caused less pathology. Accordingly, another important aspect of the present invention is that overexpression of the ***mycobacterial*** ***heat*** ***shock*** protein not only increases the immune response to that particular protein, but it also enhances the immune response to other ***mycobacterial*** proteins.

DETD [0089] The present study was designed to explore the apparent paradox that increased expression of ***heat*** ***shock*** proteins has the potential to benefit both the pathogen and the host during infection. The inventors focused on M. tuberculosis,. . . within the toxic environment of phagocytic cells, with the outcome of infection crucially dependent on the host cell-mediated immune response. ***Heat*** ***shock*** proteins were amongst the first antigens identified from M. tuberculosis.sup.7, and are currently under investigation as vaccine candidates.sup.14. The present experimental strategy was firstly to investigate the genetic basis of ***heat*** ***shock*** regulation in M. tuberculosis, and then to construct a mutant strain with a defective ***heat*** ***shock*** response. As described herein, the inventors have created novel M. tuberculosis mutants characterized by constitutive overexpression of Hsp70, and/or Hsp60,. . .

DETD [0090] Although ***mycobacterial*** ***heat*** ***shock*** proteins have been used extensively in immunological experiments, relatively little attention has been given to regulation of the ***mycobacterial*** ***heat*** ***shock*** response. As detailed in the Examples section, the present inventors have demonstrated that Hsp70 expression in M. tuberculosis is regulated. . . of only a small number of genes in M. tuberculosis, comprising the hsp70 operon and the gene encoding the ATPase ***ClpB***.sup.23,28, which like Hsp70 is preceded by an inverted repeat resembling the HAIR element.

DETD . . . M. tuberculosis .DELTA.hspR is consistent with the proposed function of Hsp70 proteins in response to stress. In contrast, overexpression of ***heat*** ***shock*** proteins in E. coli was not on its own sufficient to increase thermotolerance.sup.31.

DETD [0092] The phenotype of the .DELTA.hspR mutant during murine infection is of considerable interest. The availability of tools for ***mycobacterial*** mutagenesis has allowed identification of a number of genes involved in virulence of M. tuberculosis. Most of these mutations result. . . infection. Mutation in a cyclopropane synthetase gene interferes with lipid biosynthesis causing a change in the surface structure of the ***mycobacteria*** and affecting survival in the chronic phase.sup.35. Deletion of the gene encoding the enzyme isocitrate lyase similarly reduces persistence.sup.36. A. . .

in this case is that utilization of fatty acid derived substrates via the glyoxylate pathway makes an essential contribution to ***mycobacterial*** metabolism in the chronic phase of infection.

DETDDELTA.hspR mutant. Firstly, the high level of the Hsp70 proteins within the cell may block some developmental program involved in ***mycobacterial*** adaptation. If, for example, persistence involves formation of some spore-like `dormant` form of the organism.sup.37, it is possible that this. . . .

DETD . . . of Hsp70-specific IFN-.gamma. secreting splenocytes in comparison to wild type BCG. The enhanced immune response observed under these conditions, presents ***mycobacterial*** mutants capable of overexpressing ***heat*** ***shock*** proteins as excellent candidates for use in novel vaccines and treatments for tuberculosis..sup.1

DETD . . . production of a single-chain antibody fragment by coproduction of molecular chaperones has been observed in Bacillus subtilis.sup.38 constitutive overexpression of ***heat*** ***shock*** proteins in ***mycobacteria*** resulting in enhanced immune response has been demonstrated for the first time by the present inventors. Secretion of proteins from viable ***mycobacteria*** is thought to facilitate their early immune recognition and is used as a criterion for selection of candidate antigens for. . . the present inventors demonstrate that the effect of Hsp70 overexpression on protein secretion in vivo enhances immune responses to other ***mycobacterial*** proteins. Hsp70 released from ***mycobacterial*** cells promotes presentation of ***mycobacterial*** antigens or antigen fragments attached to its peptide-binding site. Consistent with both of the above scenarios, infection of mice with. . . .

DETD [0096] Accordingly, the enhanced immune response observed following exposure to ***mycobacterial*** mutants overexpressing ***heat*** ***shock*** proteins is not solely a result of the increase in the amount of ***heat*** ***shock*** proteins present themselves, it is also thought to be a result of the chaperone function of the ***heat*** ***shock*** protein. Therefore, functions of proteins such as Hsp70 in promoting the secretion of other ***mycobacterial*** proteins, promoting the immune presentation of other ***mycobacterial*** antigens and acting directly on immune cells inducing accessory immune signals, are also important characteristics of any ***heat*** ***shock*** protein overexpressing strain.

DETD [0097] While further analysis of the hspR mutant provides an opportunity to assess these different aspects of ***heat*** ***shock*** protein immunogenicity, the present study demonstrates that, on balance, Hsp70 overexpression favors the host over the pathogen during the chronic. . . tuberculosis infection. With an estimated one third of the global population currently infected with M tuberculosis.sup.41, interventions targeted against persistent ***mycobacteria*** could have profound public health impact. Induction of ***mycobacterial*** ***heat*** ***shock*** protein expression by specific disruption of HspR regulation or by promotion of protein denaturation, for example may provide a novel. . . .

DETD . . . with a HAIR-like sequence. Interestingly, the lead gene Rv0251c has also been shown to be under the control of the ***heat*** - ***shock*** responsive ECF sigma factor, .sigma.E, and is also prominent in response to treatment with SDS..sup.61 This dual control mechanism may account for the relatively modest elevation of Rv0251c transcription in the .DELTA.hspR mutant compared to that observed under ***heat*** ***shock*** conditions in the wild-type.

DETD [0099] Rv0251c encodes a 159 amino acid protein belonging to the small
 heat ***shock*** protein family, termed Hsp20, or the
 .alpha.-crystallin family. Its predicted size is consistent with the
 approximately 20kD protein observed by SDS-PAGE to be upregulated in the
 .DELTA.hspR.DELTA.hrcA mutant (FIG. 12B). The small ***heat***
 shock proteins, like the larger ***heat*** ***shock***
 protein families, are found widely in bacterial and eukaryotic cells and
 appear to function as molecular chaperones at least in. . . variously
 referred to as the 14kD antigen, 16kD antigen, Hsp16.3,
 .alpha.-crystallin (Acr), or HspX. This gene is not induced by
 heat ***shock***, but is upregulated in stationary phase
 cultures and during the hypoxic response..sup.51,67,77,78 It is possible
 that the different .alpha.-crystallin homologues. . .

DETD [0100] Within the .DELTA.hspR-upregulated ORF set, the Hsp70 and Acr2
 operon genes were upregulated during ***heat*** ***shock***
 along with bfrB, groES and Rv3654c. The bacterioferritin gene, bfrB, and
 Rv3654c, encoding an 8kD protein with unknown function, are not preceded
 by obvious HspR binding sites, but their coregulation with
 HAIR-associated genes in both ***heat*** ***shock*** and the
 mutant suggest an indirect link to HspR. The majority of genes
 upregulated in the mutant were neither associated with HAIR sequences
 nor were they upregulated during ***heat*** ***shock***. We
 conclude that the induction of these genes is a consequence of the
 physiological changes associated with overexpression of the
 HspR-regulated proteins and may not be directly relevant to the normal
 heat ***shock*** response. An interesting example of this
 was the trend for upregulation of ribosomal protein expression, which
 was also mirrored in. . .

DETD [0101] A surprising omission from the .DELTA.hspR upregulated list was
 clpB, which encodes another probable molecular chaperone. We
 have previously shown the elevation of ***ClpB*** expression in the
 mutant by proteomic analysis.sup.68 which suggests that the ***clpB***
 mRNA is of a sufficiently short half life to preclude detection of the
 .DELTA.hspR-associated transcriptional increase. The detection of
 substantially increased ***clpB*** mRNA in the wild-type after
 heat ***shock*** at 45.degree. C. is explained by
 upregulation of ***clpB*** transcription by the heat inducible sigma
 factor, .sigma.H, as well as release of HspR repression..sup.66

DETD . . . Though not wishing to be bound by the following theory, it is
 thought that release of HspR repression significantly influences
 heat ***shock*** protein production and may therefore have

a
 corresponding effect on the host immune system. The findings of
 heat ***shock*** protein manipulation are not limited to
 mycobacterial organisms, and may also be extrapolated to other
 infectious agents that express ***heat*** ***shock*** protein.

DETD [0104] In order to create mutants having altered expression of more than
 one ***mycobacterial*** protein a similar strategy as discussed
 above was employed to replace the hrcA gene (Rv2374c) in the .DELTA.hspR
 strains with. . .

DETD . . . suicide plasmids containing the mutated but unmarked target
 gene, hyg, sacB and LacZ. The plasmid will be introduced to the
 mycobacteria as described above and single cross-over
 integrants
 selected as hygromycin resistant (hygR), LacZ+(blue) colonies on
 hygromycin/X-gal medium. A single clone. . .

DETD . . . promoter regions. Thus, we can conclude that the HrcA repressor

acts as the main transcriptional controller of the Hsp60/GroE family
 heat ***shock*** response, with some cross-talk between the
 Hsp60 and Hsp70 responses demonstrated by the induction of GroES
 expression in the hspR. . . .

DETD . . . a conserved hypothetical protein with unknown function.
 Expression of both Rv0991c and the adjacent downstream ORF, Rv0990c, was
 elevated during ***heat*** ***shock*** but Rv0990c was not
 significantly upregulated in the mutant. Whether the two genes are
 transcribed as a bicistronic message or. . . separately regulated and
 transcribed remains to be conclusively determined. Thus, it is clear
 that HrcA regulates not just the Hsp60 ***heat*** ***shock***
 response but also Rv0991c and probably Rv0990c. In light of the effect
 of the .DELTA.hspR mutation on the virulence of. . . .

DETD . . . C. transcriptional snapshot, one skilled in the art may
 conclude that that the HspR and HrcA regulons, which dominate the
 heat ***shock*** proteome comprise only a part of the
 overall adaptive response. Genes regulated by .sigma.H and .sigma.E are
 prominent in the. . . of the .sigma.B gene suggests overlap with the
 general stress response. These different regulatory layers are
 interlinked, with hsp70 and ***clpB*** under dual HspR and .sigma.H
 control, and acr2 under dual HspR and .sigma.E control. Moreover, the
 heat inducible expression of. . . .

DETD . . . above may be employed to create mutants continuing multiple
 modifications resulting in the overexpression of more than one or two
 heat ***shock*** proteins.

DETD [0111] Therapeutics including vaccines comprising ***mycobacterial***
 mutants of the present invention, such as BCG overexpressing Hsp60
 and/or Hsp70, can be prepared in physiologically acceptable
 formulations, such. . . .

DETD . . . may be administered in combination with other compositions and
 procedures for the treatment of other disorders occurring in combination
 with ***mycobacterial*** disease. For example, tuberculosis
 frequently occurs as a secondary complication associated with acquired
 immunodeficiency syndrome (AIDS). Patients undergoing treatment AIDS. . . .

DETD . . . located using RNA extracted from cultures of BCG and the
 corresponding .DELTA.hspR mutant grown at 37.degree. C., with or without
 heat ***shock*** for 45 min at 45.degree. C., as described
 by Mangan et al..¹⁵. .gamma.[³²P]-labelled primer (PEXI,
 5'-CCTCCTGAATATGTAGAG-3') (SEQ ID NO: 14). . . .

DETD [0126] Bone marrow-derived macrophages were cultivated and infected with
 mycobacteria as previously described.⁴³ but using
 Macrophage-SFM Medium (Life Technologies) supplemented with 10 ng/ml
 IL-3 (Pharminogen, Franklin Lakes, N.J., U.S.A.).

DETD [0132] Exposure of M. tuberculosis to increased temperature results in
 elevated transcription of ***heat*** ***shock*** genes and
 expression of the corresponding proteins.^{15,16} The regulatory
 mechanisms involved have not been characterized. Two general mechanisms
 for ***heat*** ***shock*** regulation have been identified in
 bacteria. Induction of the response in E. coli involves transcriptional
 activation, with increased levels of an alternative sigma factor,
 sigma-32, directing RNA polymerase towards genes preceded by a consensus
 heat ***shock*** promoter sequence.¹⁷ In contrast, in
 Bacillus subtilis the ***heat*** ***shock*** response is
 regulated by transcriptional repression.¹⁸ In unstressed cells, the
 HrcA repressor blocks transcription by binding to an inverted repeat
 element upstream of the ***heat*** ***shock*** genes, with

repression being released in response to stress stimuli. Inspection of the genome sequence of *M. tuberculosis*.sup.19 suggests repression as the probable mechanism of ***heat*** ***shock*** regulation. Open reading frame Rv2374c encodes a homologue of the HrcA repressor, while Rv0353 encodes a protein similar to HspR, . . . in *Helicobacter pylori*.sup.21. The *M. tuberculosis* hspR is the fourth gene in an operon comprising Hsp70, followed by genes encoding ***GrpE*** and DnaJ, ***heat*** ***shock*** proteins that have functional interactions with Hsp70.sup.22 (FIG. 1a).

DETD . . . the HspR-associated inverted repeat (HAIR) identified in *Streptomyces*.sup.20,23. HspR showed no binding to a control irrelevant oligonucleotide. The effect of ***heat*** ***shock*** on the HspR-HAIR interaction was tested by carrying out the reaction at 48.degree. C. Heating had no effect on the gel shift pattern. An effect of ***heat*** ***shock*** was observed, however, when a ***mycobacterial*** extract was included in the assay. Reaction of the

oligonucleotide with HspR and the cell extract at low temperature, 30.degree.. . .

DETD . . . together form the functional repressor, with sequestration of Hsp70 as a result of binding to denatured proteins releasing repression during ***heat*** ***shock*** .sup.24.

DETD . . . in cells that had been heat shocked. In the mutant, transcription occurred from both sites even in the absence of ***heat*** ***shock*** . TSP1 and TSP2 are located 5 bases and 6 bases upstream of HAIR1 and HAIR2 respectively. While transcription from both. . .

DETD . . . constitutive overexpression of bands at 90 kDa and 45 kDa in the .DELTA.hspR mutants, again corresponding to changes induced by ***heat*** ***shock*** in the wild type. The changes in protein profile were further characterized by two-dimensional gel electrophoresis. Three protein spots were upregulated in the mutant and were identified by peptide mass fingerprinting as Hsp70, ***ClpB*** , and ***GrpE*** . DnaJ, the third ***heat*** ***shock*** protein in the hsp70 operon, has a relatively basic isoelectric point (predicted pI 8.05) and was not resolved.

DETD . . . operon. To confirm that the effects were due solely to the loss of hspR, the cloned gene was reintroduced using ***mycobacterial*** expression vectors. These experiments were unsuccessful. Plasmids constitutively expressing HspR could not be maintained in ***mycobacteria*** . Although it was possible to introduce the hspR gene into *M. tuberculosis* using the inducible acetamidase promoter.sup.6, induction of HspR. . .

DETD Dissection of the ***Heat*** ***Shock*** Response to *M. tuberculosis* Using Mutants and Microarrays

DETDmu.g/ml and kanamycin at 15 .mu.g/ml were added where appropriate. 2% sucrose was added to media for counterselection of sacB. ***Heat*** ***shock*** was performed by splitting 20 ml broth cultures at late log phase into two universal tubes and placing one tube. . .

DETD . . . Tn903. Briefly, 1.5 kb regions of DNA up and downstream of hrcA were cloned around the aph gene in the ***mycobacterial*** suicide plasmid pSMT99 to make pSMT163. This plasmid cannot replicate in ***mycobacteria*** and carries sacB for counterselection against single crossover and illegitimate integration of the plasmid. 1 .mu.g of plasmid was irradiated. . .

DETD . . . based *E.coli* plasmid which carries the aph kanamycin resistance

gene and the int gene and attP site from the L5

mycobacteriophage ..sup.69 This plasmid integrates into the chromosome in single copy by site-specific recombination at the attB site. The Hsp70 operon promoter. . . of the hsp70 promoter so as to transcriptionally fuse the ORF with its own promoter albeit without the intervening hsp70, ***grpE*** and dnaJ sequence. The resultant plasmid, pSMT168, was introduced to M. tuberculosis .DELTA.hspR by electroporation.

DETD [0162] Overview of the M. tuberculosis ***Heat*** ***Shock*** Response

DETD [0163] Previous reports have described the induction of ***heat*** ***shock*** proteins in cultures of M. tuberculosis exposed to temperatures ranging from 37-48.degree. C. for varying lengths of time, and demonstrated transcriptional regulation of selected ***heat*** ***shock*** genes..sup.65,76 These studies demonstrate a complex response, which varies with both temperature and time of exposure. To obtain an overview of the ***heat*** ***shock*** response, we used whole genome microarray analysis to generate a transcriptomic snap-shot of the changes induced by incubation at 45.degree.. . . This is displayed in the scatter plot (FIG. 10A), which shows the global nature of the transcriptional changes induced by ***heat*** ***shock*** ; the expression ratio of many genes lying away from the zero line demonstrating altered expression. A list of the 100. . . all the known members of the HspR regulon, as well as the groEL and groES genes and other previously identified ***heat*** ***shock*** inducible genes including those encoding the alternative sigma factors .sigma.B, .sigma.H and .sigma.E..sup.52,60 This set of heat-inducible genes included five. . . consensus promoter regions..sup.66 This is consistent with identification of these sigma factors as both heat-inducible genes and regulators of the ***heat*** ***shock*** response. To characterize regulation of genes encoding the major ***heat*** ***shock*** proteins, we next extended the microarray approach to analysis of mutant strains of M. tuberculosis from which predicted transcriptional repressors. . .

DETD . . . exposing a set of 49 upregulated ORFs (p<0.01) in the mutant strain, including the members of the Hsp70 operon (dnaK, ***grpE*** and dnaJ) (FIG. 14, Table 1).

DETD . . . M. tuberculosis and M. tuberculosis .DELTA.hspR. In addition to the HAIR sequences already identified upstream of the Hsp70 operon and ***clpB*** .sup.68, a HAIR-like domain was present 71 bp upstream of the start codon of Rv0251c (FIG. 12A). This gene bears similarity. . .

DETD [0167] As expected the Hsp70 operon genes along with acr2 and Rv0250c were upregulated in response to ***heat*** ***shock*** . Under the conditions used in this study, acr2 was the most heat inducible gene in the genome (FIG. 10A). Other .DELTA.hspR-regulated ORFs demonstrated to be induced under ***heat*** ***shock*** were Rv3654c, bfrB and groES. Rv3654c encodes an 8 kD protein of unknown function and bfrB encodes a bacterioferritin involved. . .

DETD [0170] ORF Rv2374c in the M. tuberculosis genome shares sequence homology with the family of ***heat*** ***shock*** repressors related to the hrcA gene of B. subtilis. To test whether this ORF is similarly involved in ***heat*** ***shock*** regulation in M. tuberculosis we undertook a deletion strategy analogous to that used to generate the .DELTA.hspR mutant, replacing hrcA. . .

DETD . . . binding site, CIRCE TTAGCACTC-N9-GAGTGCTAA (SEQ ID NO: 16).sup.56 and, as for HspR, compared the putative CIRCE locations with both the ***heat*** ***shock*** expression data and the double

mutant transcriptional profile. groEL2 is preceded by two CIRCE-like elements and groES/groEL1 by one (FIG. 12B). This confirmed the hypothesis that HrcA acts as the main regulator for the GroE/Hsp60 ***heat*** ***shock*** protein family.

- DETD . . . in the .DELTA.hspR.DELTA.hrcA mutant (FIG. 15, Table 2). Both Rv0991c and the immediately adjacent downstream gene Rv0990c were upregulated after ***heat*** ***shock*** for 30 min at 45.degree. C. in the wild-type. Although no significant change was detected in transcription of Rv0990c in. . . may be coregulated. None of the remaining .DELTA.hspR.DELTA.hrcA upregulated genes were associated with CIRCE-like elements nor were they induced under ***heat*** ***shock*** in the wild-type. Similarly to the single .DELTA.hspR mutant there was a trend for ORFs encoding ribosomal proteins to be. . .
- DETD [0174] 1. Lindquist, S. & Craig, E. A. The ***heat*** - ***shock*** proteins. Annu Rev Genet 22, 631-677 (1988).
- DETD [0177] 4. Lee, B. Y. & Horwitz, M. A. Identification of macrophage and stress-induced proteins of ***Mycobacterium*** tuberculosis. J. Clin Invest 96, 245-249 (1995).
- DETD [0181] 8. Cho, B. K. et al. A proposed mechanism for the induction of cytotoxic T lymphocyte production by ***heat*** ***shock*** fusion proteins. Immunity 12, 263-272 (2000).
- DETD [0182] 9. Suto, R. & Srivastava, P. K. A mechanism for the specific immunogenicity of ***heat*** ***shock*** protein-chaperoned peptides. Science 269, 1585-1588 (1995).
- DETD [0183] 10. Arnold-Schild, D. et al. Cutting edge: receptor-mediated endocytosis of ***heat*** ***shock*** proteins by professional antigen-presenting cells. J. Immunol 162, 3757-3760 (1999).
- DETD [0185] 12. Castellino, F. et al. Receptor-mediated uptake of Antigen/ ***Heat*** ***shock*** protein complexes results in major histocompatibility complex class I antigen presentation via two distinct processing pathways. J Exp Med 191, . . .
- DETD [0186] 13. Srivastava, P. K., Menoret, A., Basu, S., Binder, R. J. & McQuade, K. L. ***Heat*** ***shock*** proteins come of age: primitive functions acquire new roles in an adaptive world. Immunity 8, 657-665 (1998).
- DETD . . . M., Mitchison, D. A. & Butcher, P. D. An effective method of RNA extraction from bacteria refractory to disruption, including ***mycobacteria***. Nucleic Acids Res 25, 675-676 (1997).
- DETD [0189] 16. Young, D. B. & Garbe, T. R. ***Heat*** ***shock*** proteins and antigens of ***Mycobacterium*** tuberculosis. Infect Immun 59, 3086-3093 (1991).
- DETD . . . D., Erickson, J. W. & Gross, C. A. The htpR gene product of E. coli is a sigma factor for ***heat*** - ***shock*** promoters. Cell 38, 383-390 (1984).
- DETD [0191] 18. Hecker, M., Schumann, W. & Volker, U. ***Heat*** - ***shock*** and general stress response in Bacillus subtilis. Mol Microbiol 19, 417-428 (1996).
- DETD [0192] 19. Cole, S. T. et al. Deciphering the biology of ***Mycobacterium*** tuberculosis from the complete genome sequence. Nature 393, 537-544 (1998).
- DETD [0195] 22. Liberek, K., Marszalek, J., Ang, D., Georgopoulos, C. & Zylicz, M. Escherichia coli DnaJ and ***GrpE*** ***heat*** ***shock*** proteins jointly stimulate ATPase activity of DnaK. Proc Natl Acad Sci U.S.A. 88, 2874-2878 (1991).
- DETD [0196] 23. Grandvalet, C., de Crecy-Lagard, V. & Mazodier, P. The ***ClpB*** ATPase of Streptomyces albus G belongs to the HspR

heat ***shock*** regulon. Mol Microbiol 31, 521-532 (1999).
 DETD . . . 25. Pelicic, V., Reyrat, J. M. & Gicquel, B. Expression of the
 Bacillus subtilis sacB gene confers sucrose sensitivity on
 mycobacteria . J. Bacteriol 178, 1197-1199 (1996).
 DETD . . . Parish, T., Mahenthiralingam, E., Draper, P., Davis, E. O. &
 Colston, M. J. Regulation of the inducible acetamidase gene of
 Mycobacterium smegmatis. Microbiology 143, 2267-2276 (1997).
 DETD . . . E. R., Frank, A. A. & Orme, I. M. Progression of chronic
 pulmonary tuberculosis in mice aerogenically infected with virulent
 Mycobacterium tuberculosis. Tuber Lung Dis 78, 57-66 (1997).
 DETD [0201] 28. Motohashi, K., Watanabe, Y., Yohda, M. & Yoshida, M.
 Heat-inactivated proteins are rescued by the DnaK.J- ***GrpE*** set
 and ***ClpB*** chaperones. Proc Natl Acad Sci USA 96, 7184-7189
 (1999).
 DETD [0204] 31. VanBogelen, R. A., Acton, M. A. & Neidhardt, F. C. Induction
 of the ***heat*** ***shock*** regulon does not produce
 thermotolerance in Escherichia coli. Genes Dev. 1, 525-531 (1987).
 DETD . . . 32. Camacho, L. R., Ensergueix, D., Perez, E., Gicquel, B. &
 Guilhot, C. Identification of a virulence gene cluster of
 Mycobacterium tuberculosis by signature-tagged transposon
 mutagenesis. Mol Microbiol 34, 257-267 (1999).
 DETD [0206] 33. Cox, J. S., Chen, B., McNeil, M. & Jacobs, W. R., Jr. Complex
 lipid determines tissue-specific replication of ***Mycobacterium***
 tuberculosis in mice. Nature 402, 79-83 (1999).
 DETD . . . 34. Manabe, Y. C., Saviola, B. J., Sun, L., Murphy, J. R. &
 Bishai, W. R. Attenuation of virulence in ***Mycobacterium***
 tuberculosis expressing a constitutively active iron repressor. Proc
 Natl Acad Sci USA 96, 12844-12848 (1999).
 DETD . . . J. S. & Jacobs, W. R. A novel mycolic acid cyclopropane
 synthetase is required for cording, persistence, and virulence of
 Mycobacterium tuberculosis. Molecular Cell 5, 717-727 (2000).
 DETD [0209] 36. McKinney, J. D. et al. Persistence of ***Mycobacterium***
 tuberculosis in macrophages and mice requires the glyoxylate shunt
 enzyme isocitrate lyase. Nature 406, 735-738 (2000).
 DETD [0210] 37. Parrish, N. M., Dick, J. D. & Bishai, W. R. Mechanisms of
 latency in ***Mycobacterium*** tuberculosis. Trends Microbiol 6,
 107-112 (1998).
 DETD . . . Richmond, J. F. L., Suzue, K., Eisen, H. N. & Young, R. A. In
 vivo cytotoxic T lymphocyte elicitation by ***mycobacterial***
 heat ***shock*** protein 70 fusion proteins maps to a
 discrete domain and is CD4.sup.+T cell independent. J. Exp Med 191,
 403-408 (2000).
 DETD [0215] 42. Mehler, A. & Young, D. B. Biochemical and antigenic
 characterization of the ***Mycobacterium*** tuberculosis 71kD
 antigen, a member of the 70kD ***heat*** - ***shock*** protein
 family. Mol Microbiol 3, 125-130 (1989).
 DETD [0216] 43. Dussurget, O. et al. Role of ***Mycobacterium***
 tuberculosis copper-zinc superoxide dismutase. Infect Immun 69, 529-533
 (2001).
 DETD . . . Duncan, K. & Young, D. B. Alteration of a single amino acid
 residue reverses fosfomycin resistance of recombinant MurA from
 Mycobacterium tuberculosis. Microbiology 145, 3177-3184 (1999).
 DETD . . . P. E., Eichelberg, K., Mayhew, M., Rothman, J. E., Houghton, A.
 N., and Germain, R. N. (2000) Receptor-mediated uptake of Antigen/
 Heat ***shock*** protein complexes results in major
 histocompatibility complex class I antigen presentation via two distinct
 processing pathways [In Process Citation]. J.. . .

DETD . . . Primm, T. P., Jakana, J., Lee, I. H., Serysheva, I., Chiu, W., Gilbert, H. F., and Quioco, F. A. (1996) ***Mycobacterium*** tuberculosis 16-kDa antigen (Hsp16.3) functions as an oligomeric structure in vitro to suppress thermal aggregation. J. Biol Chem 271: 7218-7223.

DETD . . . S., Hamlin, N., Holroyd, S., Hornsby, T., Jagels, K., Barrell, B. G., and et al. (1998) Deciphering the biology of ***Mycobacterium*** tuberculosis from the complete genome sequence [see comments] [published erratum appears in Nature 1998 Nov 12;396(6707):190]. Nature 393: 537-544.

DETD [0224] 51. Cunningham, A. F., and Spreadbury, C. L. (1998) ***Mycobacterial*** stationary phase induced by low oxygen tension: cell wall thickening and localization of the 16-kilodalton alphacrystallin homolog. J. Bacteriol 180: . . .

DETD [0225] 52. Fernandes, N. D., Wu, Q. L., Kong, D., Puyang, X., Garg, S., and Husson, R. N. (1999) A ***mycobacterial*** extracytoplasmic sigma factor involved in survival following ***heat*** ***shock*** and oxidative stress. J. Bacteriol 181: 4266-4274.

DETD [0226] 53. Grandvalet, C., de Crecy-Lagard, V., and Mazodier, P. (1999) The ***ClpB*** ATPase of Streptomyces albus G belongs to the HspR ***heat*** ***shock*** regulon. Mol Microbiol 31:521-532.

DETD . . . Erickson, J. W., and Gross, C. A. (1984) The htpR gene product of E. coli is a sigma factor for ***heat*** - ***shock*** promoters. Cell 38: 383-390.

DETD [0229] 56. Hecker, M., Schumann, W., and Volker, U. (1996) ***Heat*** - ***shock*** and general stress response in Bacillus subtilis. Mol Microbiol 19: 417-428.

DETD . . . E., Kempell, K. E., Duncan, K., Stokes, R. W., Parish, T., and Stoker, N. G. (1999) Enhanced gene replacement in ***mycobacteria***. Microbiology 145:519-527.

DETD [0232] 59. Lee, B. Y., and Horwitz, M. A. (1995) Identification of macrophage and stress-induced proteins of ***Mycobacterium*** tuberculosis. J. Clin Invest 96: 245-249.

DETD . . . Manganelli, R., Dubnau, E., Tyagi, S., Kramer, F. R., and Smith, I. (1999) Differential expression of 10 sigma factor genes in ***Mycobacterium*** tuberculosis. Mol Microbiol 31: 715-724.

DETD [0234] 61. Manganelli, R., Voskuil, M. I., Schoolnik, G. K., and Smith, I. (2001) The ***Mycobacterium*** tuberculosis ECF sigma factor sigmaE: role in global gene expression and survival in macrophages. Mol Microbiol 41: 423-437.

DETD [0235] 62. Monahan, I., Betts, J., Banerjee, D., and Butcher, P. (2001) Differential expression of ***mycobacterial*** proteins following phagocytosis by macrophages. Microbiology 147:459-471.

DETD [0236] 63. Narberhaus, F. (1999) Negative regulation of bacterial ***heat*** ***shock*** genes. Mol Microbiol 31:1-8.

DETD [0237] 64. Parish, T., and Stoker, N. G. (1997) Development and use of a conditional antisense mutagenesis system in ***mycobacteria***. FEMS Microbiol Lett 154: 151-157.

DETD [0238] 65. Patel, B. K., Banerjee, D. K., and Butcher, P. D. (1991) Characterization of the ***heat*** ***shock*** response in ***Mycobacterium*** bovis BCG. J. Bacteriol 173: 7982-7987.

DETD . . . and Husson, R. N. (2001) The alternative sigma factor SigH regulates major components of oxidative and heat stress responses in ***Mycobacterium*** tuberculosis. J. Bacteriol 183: 6119-6125.

DETD . . . Sherman, D. R., Voskuil, M., Schnappinger, D., Liao, R., Harrell, M. I., and Schoolnik, G. K. (2001) Regulation of the ***Mycobacterium*** tuberculosis hypoxic response gene encoding

alpha-crystallin. Proc Natl Acad Sci U S A 98: 7534-7539.

DETD . . . Hussell, T., Tormay, P., O'Gaora, P., Goyal, M., Betts, J., Brown, I. N., and Young, D. B. (2001) Overexpression of ***heat*** - ***shock*** proteins reduces survival of ***Mycobacterium*** tuberculosis in the chronic phase of infection. Nature Medicine 7: 732-737.

DETD [0244] 71. Suto, R., and Srivastava, P. K. (1995) A mechanism for the specific immunogenicity of ***heat*** ***shock*** protein-chaperoned peptides. Science 269: 1585-1588.

DETD [0245] 72. Wards, B. J., and Collins, D. M. (1996) Electroporation at elevated temperatures substantially improves transformation efficiency of slow-growing ***mycobacteria***. FEMS Microbiol Lett 145: 101-105.

DETD . . . H., Imboden, P., Rane, S., Brown, P. O., and Schoolnik, G. K. (1999) Exploring drug-induced alterations in gene expression in ***Mycobacterium*** tuberculosis by microarray hybridization. Proc Natl Acad Sci U S A 96: 12833-12838.

DETD [0247] 74. Wilson M., Voskuil M., Schnappinger D., Schoolnik GK (2001) Functional genomics of ***Mycobacterium*** tuberculosis using DNA microarrays in: Methods in Molecular Medicine, vol 54: ***Mycobacterium*** tuberculosis Protocols (eds: T. Parish & N. G. Stoker) Humana Press Inc, Totowa, N. J. pp335-357.

DETD [0248] 75. Yang, H., Huang, S., Dai, H., Gong, Y., Zheng, C., and Chang, Z. (1999) The ***Mycobacterium*** tuberculosis small ***heat*** ***shock*** protein Hsp16.3 exposes hydrophobic surfaces at mild conditions: conformational flexibility and molecular chaperone activity. Protein Sci 8: 174-179.

DETD [0249] 76. Young, D. B., and Garbe, T. R. (1991) ***Heat*** ***shock*** proteins and antigens of ***Mycobacterium*** tuberculosis. Infect J. Immun 59: 3086-3093.

DETD [0250] 77. Yuan, Y., Crane, D. D., and Barry, C. E., 3rd (1996) Stationary phase-associated protein expression in ***Mycobacterium*** tuberculosis: function of the ***mycobacterial*** alphacrystallin homolog. J. Bacteriol 178: 4484-4492.

DETD . . . Q., Hickey, M. J., Sherman, D. R., and Barry, C. E., 3rd (1998) The 16 kDa alpha-crystallin (Acr) protein of ***Mycobacterium*** tuberculosis is required for growth in macrophages. Proc Natl Acad Sci U S A 95:9578-9583.

CLM What is claimed is:

1. An immunogenic composition comprising ***mycobacteria*** wherein said ***mycobacteria*** comprises ***modified*** protein ***production***.
2. The composition of claim 1, wherein the modified protein expression comprises an increase in ***heat*** ***shock*** protein production.
3. The composition of claim 2, wherein the ***heat*** ***shock*** protein is selected from the group consisting of Hsp10, Hsp40, Hsp60, Hsp70, Hsp90, ***GrpE***, ***ClpB*** and ***alpha*** - ***cystallin***.
4. The composition of claim 1, wherein the ***mycobacteria*** is selected from the group consisting of M. tuberculosis, M. avium-intracellulare, M. bovis, M. kansasii, M. fortuitum, M. chelonae, M. . . .
5. The composition of claim 1, wherein the ***mycobacteria***

comprises M. tuberculosis.

6. The composition of claim 5, wherein the ***heat*** ***shock*** protein comprises Hsp 60 or Hsp 70.

7. The composition of claim 5, wherein the ***heat*** ***shock*** protein consists of Hsp 60 and Hsp 70.

8. A human or animal comprising to said human or animal an immunogenic composition wherein said composition comprises an pathogenic organism having ***modified*** ***heat*** ***shock*** protein ***production***.

11. The method of claim 10, wherein the pathogenic organism comprises M. tuberculosis and the ***modified*** ***heat*** ***shock*** protein ***production*** comprises an increase in the production of ***heat*** ***shock*** proteins.

12. The method of claim 11, wherein the ***heat*** ***shock*** protein is selected from the group consisting of Hsp10, Hsp40, Hsp60, Hsp70, Hsp90, ***GrpE***, ***ClpB*** and ***alpha*** - ***cystallin***.

13. The method of claim 11, wherein the ***heat*** ***shock*** proteins consists of Hsp 60 and Hsp 70.

14. A method for treating ***mycobacterial*** disease comprising administering to a human or animal an immunogenic composition comprising modified ***mycobacterial*** pathogens wherein said ***mycobacterial*** pathogens have increased ***heat*** ***shock*** protein production.

15. The method of claim 14, wherein the ***mycobacterial*** disease is selected from the group consisting of tuberculosis and Crohn's disease.

16. The method of claim 15, wherein the ***heat*** ***shock*** protein is selected from the group consisting of Hsp10, Hsp40, Hsp60, Hsp70, Hsp90, ***GrpE***, ***ClpB*** and ***alpha*** - ***cystallin***.

17. The method of claim 15, wherein the ***heat*** ***shock*** protein consists of Hsp 60 and Hsp 70.

19. An immunogenic composition comprising an improved BCG vaccine wherein the vaccine comprises modified M. bovis having increased ***heat*** ***shock*** protein production.

20. The immunogenic composition of claim 19, wherein the ***heat*** ***shock*** protein is selected from the group consisting of Hsp10, Hsp40, Hsp60, Hsp70, Hsp90, ***GrpE***, ***ClpB*** and ***alpha*** - ***cystallin***.

L13 ANSWER 9 OF 9 USPATFULL on STN

AN 2000:53875 USPATFULL

TI Method of identifying compounds affecting hedgehog cholesterol transfer

IN Beachy, Philip A., Baltimore, MD, United States
 Porter, Jeffrey A., Belmont, MA, United States
 PA The Johns Hopkins University School of Medicine, United States (U.S. corporation)
 PI US 6057091 20000502
 AI US 1997-946329 19971007 (8)
 RLI Continuation-in-part of Ser. No. US 1996-729743, filed on 7 Oct 1996 which is a continuation-in-part of Ser. No. US 1995-567357, filed on 4 Dec 1995 which is a continuation-in-part of Ser. No. US 1994-349498, filed on 2 Dec 1994
 PRAI US 1997-61323P 19971002 (60)
 DT Utility
 FS Granted
 EXNAM Primary Examiner: Spector, Lorraine; Assistant Examiner: Kaufman, Claire M.
 LREP Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.
 CLMN Number of Claims: 4
 ECL Exemplary Claim: 1
 DRWN 126 Drawing Figure(s); 54 Drawing Page(s)
 LN.CNT 6997

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides two novel polypeptides, referred to as the "N" and "C" fragments of hedgehog, or N-terminal and C-terminal fragments, respectively, which are derived after specific cleavage at a G.sup.dwnarw. CF site recognized by the autoproteolytic domain in the native protein. Also included are sterol-modified hedgehog polypeptides and functional fragments thereof. Methods of identifying compositions which affect hedgehog activity based on inhibition of cholesterol modification of hedgehog protein are described.

DRWD FIG. 5 shows immunoblots showing ***heat*** ***shock*** induced expression of wild type and H329A mutant hh proteins in Drosophila embryos (A) and (B) are immunoblots developed using. . .

DRWD . . . as a .about.5-kDa species when cholesterol-modified. His.sub.6 Hh-C.sub.17 was also incubated with 46 .mu.M [.sup.3 H]cholesterol/1 mM DTT, and no cholesterol- ***modified*** ***product*** was detected by autoradiography. A cholesterol-transfer activity 1% of wildtype could have been detected by this radioassay.

DRWD . . . addition of 50 mM DTT greatly increases the amount of cleavage products and addition of cholesterol does not produce a cholesterol- ***modified*** ***product*** (.about.5-kDa species). D303A was also incubated with 46 .mu.M [.sup.3 H]cholesterol/1 mM DTT, and no cholesterol- ***modified*** ***product*** was detected by autoradiography (data not shown). A cholesterol-transfer activity 1% of wildtype could have been detected by this radioassay.

DRWD . . . PI-SceI, PI-CtrI--yeast intein endonucleases; GYRA, GYRB--DNA gyrase A and B subunits; RECA--recombinase; DNAB--replicative DNA helicase; POLC--DNA polymerase III a subunit; ***CLPP*** --endopeptidase; IF-2--translation initiation factor 2; HELI--putative helicase; RFC--replication factor C; ORF--uncharacterized open reading frame product; G6PT--glucose-6-phosphate transaminase; RPO-A', PRO-A"--DNA-dependent RNA--polymerase subunits;. . . KLBA--predicted ATPase; HO--homothallic endonuclease. Species abbreviations: CAEEL--Caenorhabditis elegans; DANRE--Danio rerio; XENLA--Xenopus laevis; Cynpy--Cynops pyrrhogaster; DROHY--Drosophila hydei; DROME--Drosophila melanogaster; CANTR--Candida tropicalis; MYCLE-- ***Mycobacterium*** leprae; MYCXE-- ***Mycobacterium*** xenopi; MYCTU-- ***Mycobacterium*** tuberculosis; PORPU--Porphyra purpurea;

SYNSP--*Synechocystis* sp; CHLEU--*Chlamydomonas*; METJA--*Methanococcus jannaschii*; PYRFU--*Pyrococcus furiosus*; PYRSP--*Pyrococcus* sp.; THELI--*Thermococcus litoralis*. Several Hh and intein sequences closely.

DETD . . . as the small subunit of RUBISCO (Coruzzi, et al., EMBO J., 3:1671-1680, 1984; Broglie, et al., Science, 224:838, 1984); or ***heat*** ***shock*** promoters, e.g., soybean hsp17.5-E or hsp17.3-B (Gurley, et al., Mol. Cell. Biol., 6:559, 1986) may be used. These constructs can. . .

DETD . . . High level expression may also be achieved using inducible promoters, including, but not limited to, the metallothionine IIA promoter and ***heat*** ***shock*** promoters.

DETD . . . stripes, embryos collected at 4 to 6 hours after egg laying (AEL) at 25.degree. C. were subjected to the following ***heat*** ***shock*** protocols prior to fixation. Embryos receiving single shocks (10 or 30 minutes at 37.degree. C.) were allowed to recover for.

DETD FIG. 5 shows that ***heat*** ***shock*** induction results in the formation of an abundant species that corresponds to U based on its mobility and its interaction. . .

DETD . . . (FIG. 6, B and C; Table 1). The difference in efficiency ranges nearly as high as threefold depending upon the ***heat*** ***shock*** regime, and these results suggest that auto-proteolysis

of the Hh protein is important for optimal activity in embryonic signaling to. . .

DETD TABLE 1

Wild-type and mutant hh activity in embryonic induction of wg expression*

	minutes of	***heat***	***shock***
10	30	10/10	30/30

hshh 1.0 +/- 0.3 (93)

1.5 +/- 0.6 (120)

2.9 +/- 0.3 (41)

2.8 +/- 0.4 (54)

hshh. . .

DETD . . . cell type when hh is expressed ubiquitously at high levels. We have reproduced suppression 3.degree. and some 4.degree. fates by ***heat*** ***shock*** induction of embryos that carry our wild-type construct (FIG. 6E), but find that the H329A mutant is unable to alter. . .

DETD For studies of signaling in imaginal discs, a thermal cycler was utilized to subject larvae carrying ***heat*** ***shock*** -inducible hh constructs to successive rounds of ***heat*** ***shock*** and recovery. The effects of temperature cycling upon expression of dpp and wg in imaginal discs was examined by monitoring. . . contrast, discs from hshh H329A and control larvae showed very little change in wg and dpp expression, even under prolonged ***heat*** ***shock*** conditions and morphological changes were never observed. (M-O) The eye phenotypes of adult control (M), hshh (N) and hshh H329A. . .

DETD . . . at least some activity in early embryonic and imaginal disc induction of wg and dpp expression; in contrast, even under ***heat*** ***shock*** conditions far more severe than those required for

effects

by the wild-type protein, the H329A mutant remained completely inert

with. . .

=> s mycobact? and (modified protein product?)
L14 1 MYCOBACT? AND (MODIFIED PROTEIN PRODUCT?)

=> d bib ab kwic

L14 ANSWER 1 OF 1 USPATFULL on STN

AN 2002:307566 USPATFULL

TI Methods and compositions for therapeutic intervention in infectious disease

IN Stewart, Graham, Walton-on-Thames, UNITED KINGDOM

O'Gaora, Peadar, London, UNITED KINGDOM

Young, Douglas, Ruislip, UNITED KINGDOM

PI US 2002172685 A1 20021121

AI US 2002-79136 A1 20020220 (10)

PRAI US 2001-269801P 20010220 (60)

US 2001-294170P 20010529 (60)

DT Utility

FS APPLICATION

LREP JOHN S. PRATT, ESQ, KILPATRICK STOCKTON, LLP, 1100 PEACHTREE STREET, SUITE 2800, ATLANTA, GA, 30309

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 15 Drawing Page(s)

LN.CNT 1922

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods and compositions for the treatment and prevention of infectious diseases are provided. In particular, efficient vaccines comprising genetically modified pathogens are provided. The vaccines generally comprise ***mycobacterial*** mutants having ***modified*** ***protein*** ***production*** capabilities. In one embodiment, the mutants overexpress heat shock protein. In a specific embodiment, the ***mycobacterial*** mutant overexpresses heat shock proteins 60 and/or 70. Also provided are modified BCG vaccines capable of overexpressing heat shock proteins 60 and/or 70.

AB . . . prevention of infectious diseases are provided. In particular, efficient vaccines comprising genetically modified pathogens are provided. The vaccines generally comprise ***mycobacterial*** mutants having ***modified*** ***protein*** ***production*** capabilities. In one embodiment, the mutants overexpress heat shock protein. In a specific embodiment, the ***mycobacterial*** mutant overexpresses heat shock proteins 60 and/or 70. Also provided are modified BCG vaccines capable of overexpressing heat shock proteins. .

SUMM . . . the invention relates to the manipulation of antigen production by infectious organisms. More particularly, the present invention comprises manipulation of ***mycobacterial*** genes resulting in the modification of heat shock protein production.

SUMM [0003] ***Mycobacterial*** infections often manifest as diseases such as tuberculosis. Human infections caused by ***mycobacteria*** have been widespread since ancient times, and tuberculosis remains a leading cause of death today. Although the incidence of the disease declined in parallel with advancing standards of living since at least the mid-nineteenth century, ***mycobacterial*** diseases still constitute a leading cause of morbidity and mortality in countries with

limited medical resources and can cause overwhelming, disseminated disease in immunocompromised patients. In spite of the efforts of numerous health organizations worldwide, the eradication of

mycobacterial diseases has never been achieved, nor is eradication imminent. Nearly one third of the world's population is infected with M.. . .

SUMM [0005] Approximately half of all patients with acquired immune deficiency syndrome (AIDS) will acquire a ***mycobacterial*** infection, with TB being an especially devastating complication. AIDS patients are at higher risks of developing clinical TB and anti-TB. .

SUMM [0006] ***Mycobacteria*** other than M. tuberculosis are increasingly found in opportunistic infections that plague the AIDS patient. Organisms from the M. avium-intracellulare complex (MAC), especially serotypes four and eight, account for 68% of the ***mycobacterial*** isolates from AIDS patients. Enormous numbers of MAC are found (up to 10^{sup.10} acid-fast bacilli per gram of tissue) and, . . .

SUMM [0007] Crohn's disease is a chronic inflammatory bowel disease characterized by transmural inflammation and granuloma formation.

Mycobacterium avium subspecies paratuberculosis (M. paratuberculosis) causes a similar disease in animals. Johnes's disease, affecting cattle, causes estimated losses of \$1.5. . .

SUMM [0008] Cattle also suffer from infection with ***Mycobacterium*** bovis which causes a disease similar to tuberculosis. Control of infection is a serious herd management concern. This infection can. .

SUMM . . . of new therapeutic agents that are effective as vaccines and as treatments for disease caused by drug resistant strains of

mycobacteria .
SUMM [0011] Although over 37 species of ***mycobacteria*** have been identified, more than 95% of all human infections are caused by six species of ***mycobacteria*** : M tuberculosis, M. avium-intracellulare, M. kansasii, M. fortuitum, M. chelonae, and M. leprae. The most prevalent ***mycobacterial*** disease in humans is tuberculosis (TB) which is caused by ***mycobacterial*** species comprising M. tuberculosis, M. bovis, or M. africanum (Merck Manual 1992). Infection is typically initiated by the inhalation of. . .

SUMM [0012] There is still no clear understanding of the factors which contribute to the virulence of ***mycobacteria*** . Many investigators have implicated lipids of the cell wall and bacterial surface as contributors to colony morphology and virulence. Evidence suggests that C-mycosides, on the surface of certain

mycobacterial cells, are important in facilitating survival of the organism within macrophages. Trehalose 6,6' dimycolate, a cord factor, has been implicated for other ***mycobacteria*** .

SUMM [0014] Diagnosis of ***mycobacterial*** infection is confirmed by the isolation and identification of the pathogen, although conventional diagnosis is based on sputum smears, chest X-ray examination (CXR), and clinical symptoms. Isolation of ***mycobacteria*** on a medium takes as long a time as four to eight weeks. Species identification takes a further two weeks. There are several other techniques for detecting ***mycobacteria*** such as the polymerase chain reaction (PCR), ***mycobacterium*** tuberculosis direct test, or amplified ***mycobacterium*** tuberculosis direct test (MTD), and detection assays that utilize radioactive labels.

SUMM . . . and many times, the results are inaccurate as false positives

are sometimes seen in subjects who have been exposed to
 mycobacteria but are healthy. In addition, instances of
 mis-diagnosis are frequent since a positive result is not observed only
 in active TB patients, but also in BCG-vaccinated persons and those who
 had been infected with ***mycobacteria*** but have not developed the
 disease. It is hard therefore, to distinguish active TB patients from
 the others, such as. . . by the tuberculin skin test. Additionally,
 the tuberculin test often produces a cross-reaction in those individuals
 who were infected with ***mycobacteria*** other than M tuberculosis
 (MOTT). Diagnosis using the skin tests currently available is frequently
 subject to error and inaccuracies.

SUMM . . . no longer consistently effective as a result of the problems
 with treatment compliance contributing to the development of drug
 resistant ***mycobacterial*** strains.

SUMM . . . infectious organism genes resulting in the modification of
 protein production are provided. Specifically, the present invention
 provides a teaching of ***mycobacterial*** genetic manipulation
 which results in an increase in heat shock protein production. The
 increase in heat shock protein production results in an enhanced immune
 response to the heat shock proteins and also other ***mycobacterial***
 proteins in general.

SUMM . . . inventors of the present invention provide for the first time a
 teaching of the use of pathogenic, and more specifically
 mycobacterial, heat shock proteins in novel vaccines and
 therapeutics. The findings of the inventors are both unobvious and
 unexpected since those. . .

SUMM [0024] The vaccination methods described herein involve the manipulation
 of ***mycobacterial*** protein production. Such proteins include,
 but are not limited to, ***mycobacterial*** heat shock proteins such
 as heat shock protein 60 (Hsp60) (GroEL1, Rv3417c:GroEL2, Rv0440), Hsp10
 (GroES, Rv3418c), Hsp70 (Rv0350), DnaJ (Hsp40,. . . comprises M.
 bovis BCG (hereafter `BCG`) vaccines capable of heat shock protein
 overexpression. In another preferred embodiment, mutant strains of
 mycobacteria or BCG overexpress more than one heat shock
 protein; such mutants include for example, strains that overexpress both
 Hsp70 and. . . Hsp60. The present invention contemplates other
 combinations of heat shock protein overexpression. The present invention
 further contemplates overexpression of other ***mycobacterial***
 proteins such as antigenic proteins found in the cell wall or secreted
 by the pathogen.

SUMM [0026] Another object of the present invention is to provide methods and
 compositions for the treatment and prevention of ***mycobacterial***
 disease such as tuberculosis.

SUMM [0027] It is another object of the present invention to provide methods
 and compositions for the treatment and prevention of
 mycobacterial disease using compositions comprising genetically
 altered ***mycobacteria*** that are capable of overexpressing
 certain proteins.

SUMM . . . present invention is to provide methods and compositions for
 the treatment and prevention of tuberculosis using compositions
 comprising genetically altered ***mycobacteria*** that overexpress
 certain proteins, wherein the proteins comprise heat shock proteins,
 cell wall proteins or other antigenic proteins secreted by. . .

SUMM . . . to provide methods and compositions for the treatment and
 prevention of tuberculosis wherein the proteins overexpressed by the
 genetically altered ***mycobacteria*** comprise Hsp60, Hsp70 and
 various combinations thereof.

SUMM [0030] Another object of the present invention is to provide compositions for vaccine formulations for the prevention of ***mycobacterial*** disease.

SUMM [0032] Yet another object of the present invention is to provide compositions for vaccine formulations for the prevention of ***mycobacterial*** disease caused by ***mycobacterial*** species comprising M. tuberculosis complex, M. avium-intracellulare, M. kansasii, M. fortuitum, M. chelonae, M. leprae, M. africanum, and M. microti. . . .

SUMM [0033] Another object of the present invention is to provide methods for the manipulation of pathogenic organisms, namely ***mycobacterial*** genes, resulting in the modification of protein production.

SUMM [0034] It is yet another object of the present invention to provide methods and compositions for production of ***mycobacterial*** mutants characterized by a defective heat shock response.

SUMM [0035] Another object of the present invention is to provide methods and compositions for production of ***mycobacterial*** mutants wherein the hspR gene of M. tuberculosis has been modified resulting in the overexpression of Hsp70.

SUMM [0036] Another object of the present invention is to provide methods and compositions for production of ***mycobacterial*** mutants wherein the hspR gene of BCG has been modified resulting in the overexpression of Hsp70.

SUMM [0037] Another object of the present invention is to provide methods and compositions for production of ***mycobacterial*** mutants wherein the hrcA gene of M. tuberculosis has been modified resulting in the overexpression of Hsp60.

SUMM [0038] It is another object of the present invention to provide methods and compositions for production of ***mycobacterial*** mutants wherein the hrcA gene of M. bovis has been modified resulting in the overexpression of Hsp60.

SUMM [0039] Yet another object of the present invention is to provide methods and compositions for production of ***mycobacterial*** mutants wherein both the hspR and hrcA genes of M. tuberculosis have been modified resulting in the overexpression of both. . . .

SUMM [0040] Another object of the present invention is to provide methods and compositions for production of ***mycobacterial*** mutants wherein both the hspR and hrcA genes of BCG have been modified resulting in the overexpression of both Hsp70,. . . .

DETD [0079] ***Mycobacterial*** infections such as those causing tuberculosis, once thought to be declining in occurrence, have rebounded and again constitute a serious. . . . threat. Areas where humans are crowded together or living in substandard housing are increasingly found to have persons infected with ***mycobacteria***. Persons who are immunocompromised are at great risk of being infected with ***mycobacteria*** and dying from such infection. In addition, the emergence of drug-resistant strains of ***mycobacteria*** has added to the treatment problems of such infected persons.

DETD [0080] Many people who are infected with ***mycobacteria*** are poor or live in areas with inadequate health care facilities. As a result of various obstacles (economical, education levels. . . . these and other individuals results in the prevalence of disease frequently compounded by the emergence of drug resistant strains of ***mycobacteria***. Effective vaccines that target various strains of ***mycobacteria*** are necessary to bring the increasing numbers of tuberculosis under control.

DETD [0081] The present invention provides methods and compositions

comprising genetically modified pathogenic organisms such as

mycobacteria for the prevention and treatment of infectious disease such as tuberculosis. More particularly, the present invention provides ***mycobacterial*** mutants capable of altered protein expression. As described herein, the protein that has altered expression may be overexpressed and may comprise any relevant ***mycobacterial*** protein, such as a cell wall protein or other antigenic protein secreted by the pathogen. Typically, the overexpressed protein is. . . shock protein such as Hsp60 or Hsp70. In an alternative embodiment of the present invention, `multiple` mutants i.e. genetically modified ***mycobacteria*** capable of altered expression of more than one protein, are also provided. In a particular embodiment, `double` mutants capable of. . .

DETD [0083] The methods and compositions of the present invention may be used for vaccinating and treating ***mycobacteria*** infection in humans as well as other animals. For example, the present invention may be particularly useful for the prevention. . .

DETD [0084] As used herein the term "tuberculosis" comprises disease states usually associated with infections caused by ***mycobacteria*** species comprising M. tuberculosis complex. ***Mycobacterial*** infections caused by ***mycobacteria*** other than M. tuberculosis (MOTT) are usually caused by ***mycobacterial*** species comprising M. avium-intracellulare, M. kansasii, M. fortuitum, M. chelonae, M. leprae, M. africanum, M. microti and M. paratuberculosis.

DETD . . . shock proteins are mainly associated with disease and that these proteins are "virulence factors" that constitute the part of the ***mycobacterial*** organism that is fundamentally responsible for disease. Contrary to current knowledge however, the present inventors have examined the role of. . . more than wildtype and caused less pathology. Accordingly, another important aspect of the present invention is that overexpression of the ***mycobacterial*** heat shock protein not only increases the immune response to that particular protein, but it also enhances the immune response to other ***mycobacterial*** proteins.

DETD [0090] Although ***mycobacterial*** heat shock proteins have been used extensively in immunological experiments, relatively little attention has been given to regulation of the ***mycobacterial*** heat shock response. As detailed in the Examples section, the present inventors have demonstrated that Hsp70 expression in M. tuberculosis. .

DETD [0092] The phenotype of the .DELTA.hspR mutant during murine infection is of considerable interest. The availability of tools for ***mycobacterial*** mutagenesis has allowed identification of a

number

of genes involved in virulence of M. tuberculosis. Most of these mutations result. . . infection. Mutation in a cyclopropane synthetase gene interferes with lipid biosynthesis causing a change in the surface structure of the ***mycobacteria*** and affecting survival in the chronic phase.sup.35. Deletion of the gene encoding the enzyme isocitrate lyase similarly reduces persistence.sup.36. A. . . in this case is that utilization of fatty acid derived substrates via the glyoxylate pathway makes an essential contribution to

DETD ***mycobacterial*** metabolism in the chronic phase of infection. . . .DELTA.hspR mutant. Firstly, the high level of the Hsp70 proteins within the cell may block some developmental program involved in ***mycobacterial*** adaptation. If, for example, persistence involves formation of some spore-like `dormant` form of the

organism.sup.37, it is possible that this. . .

DETD . . . of Hsp70-specific IFN-.gamma. secreting splenocytes in comparison to wild type BCG. The enhanced immune response observed under these conditions, presents ***mycobacterial*** mutants capable of overexpressing heat shock proteins as excellent candidates for use in novel vaccines and treatments for tuberculosis..sup.1

DETD . . . antibody fragment by coproduction of molecular chaperones has been observed in Bacillus subtilis.sup.38 constitutive overexpression of heat shock proteins in ***mycobacteria*** resulting in enhanced immune response has been demonstrated for the first time by the present inventors. Secretion of proteins from viable ***mycobacteria*** is thought to facilitate their early immune recognition and is used as a criterion for selection of candidate antigens for. . . the present inventors demonstrate that the effect of Hsp70 overexpression on protein secretion in vivo enhances immune responses to other

mycobacterial proteins. Hsp70 released from

mycobacterial cells promotes presentation of

mycobacterial antigens or antigen fragments attached to its peptide-binding site. Consistent with both of the above scenarios, infection of mice with. . .

DETD [0096] Accordingly, the enhanced immune response observed following exposure to ***mycobacterial*** mutants overexpressing heat shock proteins is not solely a result of the increase in the amount of heat shock proteins. . . chaperone function of the heat shock protein. Therefore, functions of proteins such as Hsp70 in promoting the secretion of other ***mycobacterial*** proteins, promoting the immune presentation of other ***mycobacterial*** antigens and acting directly on immune cells inducing accessory immune signals, are also important characteristics of any heat shock protein. . .

DETD . . . tuberculosis infection. With an estimated one third of the global population currently infected with M tuberculosis.sup.41, interventions targeted against persistent ***mycobacteria*** could have profound public health impact. Induction of ***mycobacterial*** heat shock protein expression by specific disruption of HspR regulation or by promotion of protein denaturation, for example may provide. . .

DETD . . . have a corresponding effect on the host immune system. The findings of heat shock protein manipulation are not limited to ***mycobacterial*** organisms, and may also be extrapolated to other infectious agents that express heat shock protein.

DETD [0104] In order to create mutants having altered expression of more than one ***mycobacterial*** protein a similar strategy as discussed above was employed to replace the hrcA gene (Rv2374c) in the .DELTA.hspR strains with. . .

DETD . . . suicide plasmids containing the mutated but unmarked target gene, hyg, sacB and LacZ. The plasmid will be introduced to the ***mycobacteria*** as described above and single cross-over integrants

selected as hygromycin resistant (hygR), LacZ+(blue) colonies on hygromycin/X-gal medium. A single clone. . .

DETD [0111] Therapeutics including vaccines comprising ***mycobacterial*** mutants of the present invention, such as BCG overexpressing Hsp60 and/or Hsp70, can be prepared in physiologically acceptable formulations, such. . .

DETD . . . may be administered in combination with other compositions and procedures for the treatment of other disorders occurring in combination with ***mycobacterial*** disease. For example, tuberculosis frequently occurs as a secondary complication associated with acquired

immunodeficiency syndrome (AIDS). Patients undergoing treatment AIDS. .

- DETD [0126] Bone marrow-derived macrophages were cultivated and infected with ***mycobacteria*** as previously described.sup.43 but using Macrophage-SFM Medium (Life Technologies) supplemented with 10 ng/ml IL-3 (Pharmingen, Franklin Lakes, N.J., U.S.A.).
- DETD . . . C. Heating had no effect on the gel shift pattern. An effect of heat shock was observed, however, when a ***mycobacterial*** extract was included in the assay. Reaction of the oligonucleotide with HspR and the cell extract at low temperature, 30.degree.. . .
- DETD . . . operon. To confirm that the effects were due solely to the loss of hspR, the cloned gene was reintroduced using ***mycobacterial*** expression vectors. These experiments were unsuccessful. Plasmids constitutively expressing HspR could not be maintained in ***mycobacteria***. Although it was possible to introduce the hspR gene into M. tuberculosis using the inducible acetamidase promoter.sup.6, induction of HspR. . .
- DETD . . . Tn903. Briefly, 1.5 kb regions of DNA up and downstream of hrca were cloned around the aph gene in the ***mycobacterial*** suicide plasmid pSMT99 to make pSMT163. This plasmid cannot replicate in ***mycobacteria*** and carries sacB for counterselection against single crossover and illegitimate integration of the plasmid. 1 .mu.g of plasmid was irradiated. . .
- DETD . . . based E.coli plasmid which carries the aph kanamycin resistance gene and the int gene and attP site from the L5 ***mycobacteriophage*** .sup.69 This plasmid integrates into the chromosome in single copy by site-specific recombination at the attB site. The Hsp70 operon promoter. . .
- DETD [0177] 4. Lee, B. Y. & Horwitz, M. A. Identification of macrophage and stress-induced proteins of ***Mycobacterium*** tuberculosis. J. Clin Invest 96, 245-249 (1995).
- DETD . . . M., Mitchison, D. A. & Butcher, P. D. An effective method of RNA extraction from bacteria refractory to disruption, including ***mycobacteria***. Nucleic Acids Res 25, 675-676 (1997).
- DETD [0189] 16. Young, D. B. & Garbe, T. R. Heat shock proteins and antigens of ***Mycobacterium*** tuberculosis. Infect Immun 59, 3086-3093 (1991).
- DETD [0192] 19. Cole, S. T. et al. Deciphering the biology of ***Mycobacterium*** tuberculosis from the complete genome sequence. Nature 393, 537-544 (1998).
- DETD . . . 25. Pelicic, V., Reytrat, J. M. & Gicquel, B. Expression of the Bacillus subtilis sacB gene confers sucrose sensitivity on ***mycobacteria***. J. Bacteriol 178, 1197-1199 (1996).
- DETD . . . Parish, T., Mahenthiralingam, E., Draper, P., Davis, E. O. & Colston, M. J. Regulation of the inducible acetamidase gene of ***Mycobacterium*** smegmatis. Microbiology 143, 2267-2276 (1997).
- DETD . . . E. R., Frank, A. A. & Orme, I. M. Progression of chronic pulmonary tuberculosis in mice aerogenically infected with virulent ***Mycobacterium*** tuberculosis. Tuber Lung Dis 78, 57-66 (1997).
- DETD . . . 32. Camacho, L. R., Ensergueix, D., Perez, E., Gicquel, B. & Guilhot, C. Identification of a virulence gene cluster of ***Mycobacterium*** tuberculosis by signature-tagged transposon mutagenesis. Mol Microbiol 34, 257-267 (1999).
- DETD [0206] 33. Cox, J. S., Chen, B., McNeil, M. & Jacobs, W. R., Jr. Complex lipid determines tissue-specific replication of ***Mycobacterium*** tuberculosis in mice. Nature 402, 79-83 (1999).
- DETD . . . 34. Manabe, Y. C., Saviola, B. J., Sun, L., Murphy, J. R. &

Bishai, W. R. Attenuation of virulence in ***Mycobacterium*** tuberculosis expressing a constitutively active iron repressor. *Proc Natl Acad Sci USA* 96, 12844-12848 (1999).

DETD . . . J. S. & Jacobs, W. R. A novel mycolic acid cyclopropane synthetase is required for cording, persistence, and virulence of ***Mycobacterium*** tuberculosis. *Molecular Cell* 5, 717-727 (2000).

DETD [0209] 36. McKinney, J. D. et al. Persistence of ***Mycobacterium*** tuberculosis in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. *Nature* 406, 735-738 (2000).

DETD [0210] 37. Parrish, N. M., Dick, J. D. & Bishai, W. R. Mechanisms of latency in ***Mycobacterium*** tuberculosis. *Trends Microbiol* 6, 107-112 (1998).

DETD . . . Richmond, J. F. L., Suzue, K., Eisen, H. N. & Young, R. A. In vivo cytotoxic T lymphocyte elicitation by ***mycobacterial*** heat shock protein 70 fusion proteins maps to a discrete domain and is CD4.sup.+T cell independent. *J. Exp Med* 191, . . .

DETD [0215] 42. Mehlert, A. & Young, D. B. Biochemical and antigenic characterization of the ***Mycobacterium*** tuberculosis 71kD antigen, a member of the 70kD heat-shock protein family. *Mol Microbiol* 3, 125-130 (1989).

DETD [0216] 43. Dussurget, O. et al. Role of ***Mycobacterium*** tuberculosis copper-zinc superoxide dismutase. *Infect Immun* 69, 529-533 (2001).

DETD . . . Duncan, K. & Young, D. B. Alteration of a single amino acid residue reverses fosfomycin resistance of recombinant MurA from ***Mycobacterium*** tuberculosis. *Microbiology* 145, 3177-3184 (1999).

DETD . . . Primm, T. P., Jakana, J., Lee, I. H., Serysheva, I., Chiu, W., Gilbert, H. F., and Quijcho, F. A. (1996) ***Mycobacterium*** tuberculosis 16-kDa antigen (Hsp16.3) functions as an oligomeric structure in vitro to suppress thermal aggregation. *J. Biol Chem* 271: 7218-7223.

DETD . . . S., Hamlin, N., Holroyd, S., Hornsby, T., Jagels, K., Barrell, B. G., and et al. (1998) Deciphering the biology of ***Mycobacterium*** tuberculosis from the complete genome sequence [see comments] [published erratum appears in *Nature* 1998 Nov 12;396(6707):190]. *Nature* 393: 537-544.

DETD [0224] 51. Cunningham, A. F., and Spreadbury, C. L. (1998) ***Mycobacterial*** stationary phase induced by low oxygen tension: cell wall thickening and localization of the 16-kilodalton alpha-crystallin homolog. *J. Bacteriol* 180: . . .

DETD [0225] 52. Fernandes, N. D., Wu, Q. L., Kong, D., Puyang, X., Garg, S., and Husson, R. N. (1999) A ***mycobacterial*** extracytoplasmic sigma factor involved in survival following heat shock and oxidative stress. *J. Bacteriol* 181: 4266-4274.

DETD . . . E., Kempell, K. E., Duncan, K., Stokes, R. W., Parish, T., and Stoker, N. G. (1999) Enhanced gene replacement in ***mycobacteria***. *Microbiology* 145:519-527.

DETD [0232] 59. Lee, B. Y., and Horwitz, M. A. (1995) Identification of macrophage and stress-induced proteins of ***Mycobacterium*** tuberculosis. *J. Clin Invest* 96: 245-249.

DETD . . . Manganelli, R., Dubnau, E., Tyagi, S., Kramer, F. R., and Smith, I. (1999) Differential expression of 10 sigma factor genes in ***Mycobacterium*** tuberculosis. *Mol Microbiol* 31: 715-724.

DETD [0234] 61. Manganelli, R., Voskuil, M. I., Schoolnik, G. K., and Smith, I. (2001) The ***Mycobacterium*** tuberculosis ECF sigma factor sigmaE: role in global gene expression and survival in macrophages. *Mol Microbiol* 41: 423-437.

DETD [0235] 62. Monahan, I., Betts, J., Banerjee, D., and Butcher, P. (2001) Differential expression of ***mycobacterial*** proteins following phagocytosis by macrophages. Microbiology 147:459-471.

DETD [0237] 64. Parish, T., and Stoker, N. G. (1997) Development and use of a conditional antisense mutagenesis system in ***mycobacteria***. FEMS Microbiol Lett 154: 151-157.

DETD [0238] 65. Patel, B. K., Banerjee, D. K., and Butcher, P. D. (1991) Characterization of the heat shock response in ***Mycobacterium*** bovis BCG. J. Bacteriol 173: 7982-7987.

DETD . . . and Husson, R. N. (2001) The alternative sigma factor SigH regulates major components of oxidative and heat stress responses in ***Mycobacterium*** tuberculosis. J. Bacteriol 183: 6119-6125.

DETD . . . Sherman, D. R., Voskuil, M., Schnappinger, D., Liao, R., Harrell, M. I., and Schoolnik, G. K. (2001) Regulation of the ***Mycobacterium*** tuberculosis hypoxic response gene encoding alpha-crystallin. Proc Natl Acad Sci U S A 98: 7534-7539.

DETD . . . P., Goyal, M., Betts, J., Brown, I. N., and Young, D. B. (2001) Overexpression of heat-shock proteins reduces survival of ***Mycobacterium*** tuberculosis in the chronic phase of infection. Nature Medicine 7: 732-737.

DETD [0245] 72. Wards, B. J., and Collins, D. M. (1996) Electroporation at elevated temperatures substantially improves transformation efficiency of slow-growing ***mycobacteria***. FEMS Microbiol Lett 145: 101-105.

DETD . . . H., Imboden, P., Rane, S., Brown, P. O., and Schoolnik, G. K. (1999) Exploring drug-induced alterations in gene expression in ***Mycobacterium*** tuberculosis by microarray hybridization. Proc Natl Acad Sci U S A 96: 12833-12838.

DETD [0247] 74. Wilson M., Voskuil M., Schnappinger D., Schoolnik GK (2001) Functional genomics of ***Mycobacterium*** tuberculosis using DNA microarrays in: Methods in Molecular Medicine, vol 54: ***Mycobacterium*** tuberculosis Protocols (eds: T. Parish & N. G. Stoker) Humana Press Inc, Totowa, N. J. pp335-357.

DETD [0248] 75. Yang, H., Huang, S., Dai, H., Gong, Y., Zheng, C., and Chang, Z. (1999) The ***Mycobacterium*** tuberculosis small heat shock protein Hsp16.3 exposes hydrophobic surfaces at mild conditions: conformational flexibility and molecular chaperone activity. Protein Sci. . . .

DETD [0249] 76. Young, D. B., and Garbe, T. R. (1991) Heat shock proteins and antigens of ***Mycobacterium*** tuberculosis. Infect J. Immun 59: 3086-3093.

DETD [0250] 77. Yuan, Y., Crane, D. D., and Barry, C. E., 3rd (1996) Stationary phase-associated protein expression in ***Mycobacterium*** tuberculosis: function of the ***mycobacterial*** alphacrystallin homolog. J. Bacteriol 178: 4484-4492.

DETD . . . Q., Hickey, M. J., Sherman, D. R., and Barry, C. E., 3rd (1998) The 16 kDa alpha-crystallin (Acr) protein of ***Mycobacterium*** tuberculosis is required for growth in macrophages. Proc Natl Acad Sci U S A 95:9578-9583.

CLM What is claimed is:

1. An immunogenic composition comprising ***mycobacteria*** wherein said ***mycobacteria*** comprises ***modified*** ***protein*** ***production*** .

4. The composition of claim 1, wherein the ***mycobacteria*** is selected from the group consisting of M. tuberculosis, M. avium-intracellulare, M. bovis, M. kansasii, M. fortuitum, M. chelonae,

M.. . .

5. The composition of claim 1, wherein the ***mycobacteria*** comprises M. tuberculosis.

14. A method for treating ***mycobacterial*** disease comprising administering to a human or animal an immunogenic composition comprising modified ***mycobacterial*** pathogens wherein said ***mycobacterial*** pathogens have increased heat shock protein production.

15. The method of claim 14, wherein the ***mycobacterial*** disease is selected from the group consisting of tuberculosis and Crohn's disease.

=> s mycobacter? (2w) transformed

L15 97 MYCOBACTER? (2W) TRANSFORMED

=> dup rem l15

PROCESSING COMPLETED FOR L15

L16 65 DUP REM L15 (32 DUPLICATES REMOVED)

=> d bib ab 1-

YOU HAVE REQUESTED DATA FROM 65 ANSWERS - CONTINUE? Y/(N):y

L16 ANSWER 1 OF 65 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1

AN 2003:355483 BIOSIS

DN PREV200300355483

TI whmD, an essential cell division gene from mycobacteria.

AU Bishai, William R. (1); Gomez, James E.

CS (1) Baltimore, MD, USA USA

ASSIGNEE: Johns Hopkins University

PI US 6590087 July 08, 2003

SO Official Gazette of the United States Patent and Trademark Office Patents,
(July 8 2003) Vol. 1272, No. 2, pp. No Pagination.

<http://www.uspto.gov/web/menu/patdata.html>. e-file.

ISSN: 0098-1133.

DT Patent

LA English

AB A whmD gene that controls sporulation in ***mycobacteria***, vectors
and ***transformed*** cells containing the gene.

L16 ANSWER 2 OF 65 USPATFULL on STN

AN 2003:146354 USPATFULL

TI Insertional mutations in mycobacteria

IN Jacobs, William R., JR., City Island, NY, UNITED STATES

Bloom, Barry, Hastings-on-Hudson, NY, UNITED STATES

Kalpana, Ganjam V., Yonkers, NY, UNITED STATES

Cirillo, Jeffrey D., Mountain View, CA, UNITED STATES

McAdam, Ruth, Near Hatfield, UNITED KINGDOM

PI US 2003100100 A1 20030529

AI US 2001-898762 A1 20010703 (9)

RLI Continuation of Ser. No. US 1997-850977, filed on 5 May 1997, PENDING

Continuation of Ser. No. US 1994-247711, filed on 23 May 1994, ABANDONED

Continuation-in-part of Ser. No. US 1994-190240, filed on 1 Feb 1994,
ABANDONED Continuation of Ser. No. US 1991-806706, filed on 12 Dec 1991,
ABANDONED Continuation-in-part of Ser. No. US 1991-714656, filed on 13
Jun 1991, ABANDONED

DT Utility
FS APPLICATION
LREP Craig J. Arnold, Amster, Rothstein & Ebenstein, 90 Park Avenue, New
York, NY, 10016
CLMN Number of Claims: 30
ECL Exemplary Claim: 1
DRWN 29 Drawing Page(s)
LN.CNT 1691

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A mutated mycobacterium selected from the class consisting of mutated M.
bovis-BCG, mutated M. tuberculosis, and mutated M. leprae. The mutation
of M. bovis-BCG, M. tubeiculosis, or M. leprae is preferably effected
through an insertional mutation of a mycobacterial gene. The insertional
mutagenesis may be effected, for example, through illegitimate
recombination or by a mycobacterial transposon. Such mutated
mycobacteria may then be transformed with an expression vector(s)
containing a complement gene to the gene which is mutated, and
preferably also including a heterologous gene.

L16 ANSWER 3 OF 65 USPATFULL on STN

AN 2003:134810 USPATFULL

TI Polynucleotide functionally coding for the LHP protein from
Mycobacterium tuberculosis, its biologically active derivative
fragments, as well as methods using the same

IN Gicquel, Brigitte, Paris, FRANCE
Berthet, Francois-Xavier, Paris, FRANCE
Anderson, Peter, Bronshoj, DENMARK
Rasmussen, Peter Birk, Bergsgade, DENMARK

PA INSTITUT PASTEUR, Paris Cedex, FRANCE (non-U.S. corporation)

PI US 2003092899 A1 20030515

AI US 2002-140045 A1 20020508 (10)

RLI Division of Ser. No. US 1998-116492, filed on 16 Jul 1998, GRANTED, Pat.
No. US 6436409

PRAI US 1997-52631P 19970716 (60)

DT Utility

FS APPLICATION

LREP OBLON SPIVAK MCCLELLAND MAIER & NEUSTADT PC, FOURTH FLOOR, 1755
JEFFERSON DAVIS HIGHWAY, ARLINGTON, VA, 22202

CLMN Number of Claims: 55

ECL Exemplary Claim: 1

DRWN 14 Drawing Page(s)

LN.CNT 2572

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is directed to a polynucleotide carrying an open
reading frame coding for an antigenic polypeptide from Mycobacterium
tuberculosis, named lhp, which is placed under the control of its own
regulation signals which are functional in mycobacteria, specially in
mycobacteria belonging to the Mycobacterium tuberculosis complex and
also in fast growing mycobacteria such as Mycobacterium smegmatis. The
invention is also directed to the polypeptide LHP encoded by lhp and
most preferably to suitable antigenic portions of LHP as well as to
oligomeric polypeptides containing more than one unit of LHP or an
antigenic portion of LHP. The invention concerns also immunogenic and

vaccine compositions containing a polypeptide or an oligomeric polypeptide such as defined above, as well as antibodies directed specifically against such polypeptides that are useful as diagnostic reagents. In another embodiment, the present invention is directed to a polynucleotide carrying the natural regulation signals of lhp which is useful in order to express heterologous proteins in mycobacteria. Finally, the present invention is directed to oligonucleotides comprising at least 12 consecutive nucleotides from the regulation sequence of lhp which are useful as reagents for detecting the presence of Mycobacterium tuberculosis in a biological sample.

L16 ANSWER 4 OF 65 USPATFULL on STN

AN 2003:136957 USPATFULL

TI Insertional mutations in mycobacteria

IN Jacobs, Jr., William R., City Island, NY, United States

Bloom, Barry, Hastings-on-Hudson, NY, United States

Kalpana, Ganjam V., Yonkers, NY, United States

Cirillo, Jeffrey D., Mountain View, CA, United States

McAdam, Ruth, Essendon, UNITED KINGDOM

PA Albert Einstein College of Medicine of Yeshiva University, Bronx, NY, United States (U.S. corporation)

PI US 6566121 B1 20030520

AI US 1997-850977 19970505 (8)

RLI Continuation of Ser. No. US 1994-247711, filed on 23 May 1994, now abandoned Continuation-in-part of Ser. No. US 1994-190240, filed on 1 Feb 1994, now abandoned Continuation of Ser. No. US 1991-806706, filed on 12 Dec 1991, now abandoned Continuation-in-part of Ser. No. US 1991-714656, filed on 13 Jun 1991, now abandoned

DT Utility

FS GRANTED

EXNAM Primary Examiner: Swartz, Rodney P

LREP Amster, Rothstein & Ebenstein

CLMN Number of Claims: 4

ECL Exemplary Claim: 1

DRWN 40 Drawing Figure(s); 29 Drawing Page(s)

LN.CNT 1746

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A mutated mycobacterium selected from the class consisting of mutated M.bovis-BCG, mutated M.tuberculosis, and mutated M. leprae. The mutation of M.bovis-BCG, M.tuberculosis, or M. leprae is preferably effected through an insertional mutation of a mycobacterial gene. The insertional mutagenesis may be effected, for example, through illegitimate recombination or by a mycobacterial transposon. Such mutated mycobacteria may then be transformed with an expression vector(s) containing a complement gene to the gene which is mutated, and preferably also including a heterologous gene.

L16 ANSWER 5 OF 65 USPATFULL on STN

AN 2003:67565 USPATFULL

TI Recombinant polypeptides and peptides, nucleic acids coding for the same and use of these polypeptides and peptides in the diagnostic of tuberculosis

IN Content, Jean, Rhode St-Genese, BELGIUM

De Wit, Lucas, Puurs, BELGIUM

De Bruyn, Jacqueline, Beersel, BELGIUM

Van Vooren, Jean-Paul, St-Pieters Leeuw, BELGIUM

PA N.V. Innogenetics S.A., BELGIUM (non-U.S. corporation)

PI US 6531138 B1 20030311
AI US 1999-342673 19990629 (9)
RLI Continuation of Ser. No. US 1995-447430, filed on 22 May 1995, now patented, Pat. No. US 5916558 Continuation of Ser. No. US 1991-690949, filed on 8 Jul 1991, now abandoned
PRAI GB 1989-402571 19890919
DT Utility
FS GRANTED
EXNAM Primary Examiner: Swartz, Rodney P
LREP Fish & Richardson P.C.
CLMN Number of Claims: 18
ECL Exemplary Claim: 1
DRWN 62 Drawing Figure(s); 60 Drawing Page(s)
LN.CNT 4103

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to recombinant polypeptides and peptides and particularly to the polypeptide containing in its polypeptidic chain the following amino acid sequence: the one extending from the extremity constituted by nucleotide at position (1) to the extremity constituted by nucleotide at position (194) represented in FIG. 4a and FIG. 4b. The polypeptides and peptides of the invention can be used for the diagnostic of tuberculosis, and can also be part of the active principle in the preparation of vaccine against tuberculosis.

L16 ANSWER 6 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2003:378221 CAPLUS

DN 139:64149

TI Molecular cloning and sequencing of the merozoite surface antigen 2 gene from Plasmodium falciparum strain FCC-1/HN and expression of the gene in mycobacteria

AU Zheng, Chunfu; Xie, Peimei; Chen, Yatang

CS Institute of Infectious and Parasitic Diseases, The First Affiliated Hospital of Chongqing Medical University, Chungking, 400016, Peop. Rep. China

SO Journal of Eukaryotic Microbiology (2003), 50(2), 140-143

CODEN: JEMIED; ISSN: 1066-5234

PB Society of Protozoologists

DT Journal

LA English

AB Strain bacillus Calmette-Guerin (BCG) of Mycobacterium bovis has been used as a live bacterial vaccine to immunize more than 3 billion people against tuberculosis. In an attempt to use this vaccine strain as a vehicle for protective antigens, the gene encoding merozoite surface antigen 2 (MSA2) was amplified from strain FCC-1/HN Plasmodium falciparum genome, sequenced, and expressed in M. bovis BCG under the control of an expression cassette carrying the promoter of heat shock protein 70 (HSP70) from Mycobacterium tuberculosis. The recombinant shuttle plasmid pBCG/MSA2 was introduced into mycobacteria by electroporation, and the recombinant mycobacteria harboring pBCG/MSA2 could be induced by heating to express MSA2; the mol. mass of recombinant MSA2 was about 31 kDa. This first report of expression of the full-length P. falciparum MSA2 gene in BCG provides evidence for use of the HSP70 promoter in expressing a foreign gene in BCG and in development of BCG as a multivalent vectoral vaccine for malaria.

RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 7 OF 65 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 2
 AN 2002:293976 BIOSIS
 DN PREV200200293976
 TI Recombinant mycobacteria.
 AU Bloom, Barry R. (1); Davis, Ronald W.; Jacobs, William R., Jr.; Young,
 Richard A.; Husson, Robert N.
 CS (1) Hastings on Hudson, NY USA
 ASSIGNEE: Albert Einstein College of Medicine of Yeshiva University
 PI US 6372478 April 16, 2002
 SO Official Gazette of the United States Patent and Trademark Office Patents,
 (Apr. 16, 2002) Vol. 1257, No. 3, pp. No Pagination.
<http://www.uspto.gov/web/menu/patdata.html>. e-file.
 ISSN: 0098-1133.
 DT Patent
 LA English
 AB Recombinant mycobacterial vaccine vehicles capable of expressing DNA of
 interest which encodes at least one protein antigen for at least one
 pathogen against which an immune response is desired and which can be
 incorporated into the mycobacteria or stably integrated into the
 mycobacterial genome. The vaccine vehicles are useful for administration
 to mammalian hosts for purposes of immunization. A recombinant vector
 which replicates in E. coli but not in mycobacteria is also disclosed. The
 recombinant vector includes 1) a mycobacterial gene or portions thereof,
 necessary for recombination with homologous sequences in the genome of
 mycobacteria ***transformed*** with the recombinant plasmid;
 2) all or a portion of a gene which encodes a polypeptide or protein whose
 expression is desired in ***mycobacteria*** ***transformed*** with
 the recombinant plasmid; 3) DNA sequences necessary for replication and
 selection in E coli; and 4) DNA sequences necessary for selection in
 mycobacteria (e.g., drug resistance). The present invention also relates
 to two types of recombinant vectors useful in introducing DNA of interest
 into mycobacteria, where it is expressed. One type of vector is a
 recombinant phasmid capable of replicating as a plasmid in E. coli and of
 lysogenizing a mycobacterial host. The other type of vector is a
 recombinant plasmid which can be introduced into mycobacteria, where it is
 stably maintained extrachromosomally.

L16 ANSWER 8 OF 65 USPATFULL on STN
 AN 2002:272887 USPATFULL
 TI IniB, iniA and iniC genes of mycobacteria and methods of use
 IN Alland, David, Dobbs Ferry, NY, UNITED STATES
 Bloom, Barry R., Hastings-on-Hudson, NY, UNITED STATES
 Jacobs, William R., JR., City Island, NY, UNITED STATES
 PI US 2002151008 A1 20021017
 AI US 2001-918951 A1 20010731 (9)
 RLI Continuation of Ser. No. US 1998-177349, filed on 23 Oct 1998, PATENTED
 DT Utility
 FS APPLICATION
 LREP Elie H. Gendloff, Ph.D., Esq., AMSTER, ROTHSTEIN & EBENSTEIN, 90 Park
 Avenue, New York, NY, 10016
 CLMN Number of Claims: 47
 ECL Exemplary Claim: 1
 DRWN 10 Drawing Page(s)
 LN.CNT 935
 CAS INDEXING IS AVAILABLE FOR THIS PATENT.
 AB This invention relates to the identification, cloning, sequencing and

characterization of the iniB, iniA and iniC genes of mycobacteria which are induced by a broad class of antibiotics that act by inhibiting cell wall biosynthesis, including the first line antituberculosis agents, isoniazid and ethambutol. The present invention provides purified and isolated iniB, iniA, iniC and iniB promoter nucleic acids which may comprise the iniBAC operon, as well as mutated forms of these nucleic acids. The present invention also provides one or more single-stranded nucleic acid probes which specifically hybridize to the iniB, iniA, iniC and iniB promoter nucleic acids, and mixtures thereof, which may be formulated in kits, and used in the diagnosis of drug-resistant mycobacterial strain. The present invention also provides methods for the screening and identification of drugs effective against Mycobacterium tuberculosis using induction of the iniB promoter.

L16 ANSWER 9 OF 65 USPATFULL on STN

AN 2002:283166 USPATFULL

TI Shuttle vectors for the introduction of DNA into mycobacteria and utilization of such bacteria as vaccines

IN Escuyer, Vincent, Massy, FRANCE
Baulard, Alain, Tournai, BELGIUM
Berche, Patrick, Saint-Cloud, FRANCE
Locht, Camille, Wannehain, FRANCE
Haddad, Nadia, Paris, FRANCE

PA Institut National de la Sante et de la Recherche Medicale (Inserm),
Paris, FRANCE (non-U.S. corporation)
Institut Pasteur de Lille, Lille Cedex, FRANCE (non-U.S. corporation)

PI US 6472213 B1 20021029

AI US 1999-468543 19991221 (9)

RLI Division of Ser. No. US 737588, now patented, Pat. No. US 6074866

PRAI FR 1994-6202 19940520

DT Utility

FS GRANTED

EXNAM Primary Examiner: McGarry, Sean; Assistant Examiner: Zara, J.

LREP Greenblum & Bernstein, P.L.C.

CLMN Number of Claims: 4

ECL Exemplary Claim: 1

DRWN 9 Drawing Figure(s); 9 Drawing Page(s)

LN.CNT 559

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Shuttle vectors for inserting DNA in mycobacteria including at least one origin of functional replication in said mycobacteria, another origin of functional replication in other bacteria, an enzyme cutting site allowing the insertion of DNA coding for a protein capable of being expressed in the mycobacteria, wherein the shuttle vectors also carry a gene providing on said mycobacteria resistance to a compound containing a heavy metal.

L16 ANSWER 10 OF 65 USPATFULL on STN

AN 2002:268879 USPATFULL

TI Compositions and methods of their use in the treatment, prevention and diagnosis of tuberculosis

IN Skeiky, Yasir, Seattle, WA, United States

PA Corixa Corporation, Seattle, WA, United States (U.S. corporation)

PI US 6465633 B1 20021015

AI US 1999-470191 19991223 (9)

PRAI US 1998-113952P 19981224 (60)

DT Utility

FS GRANTED

EXNAM Primary Examiner: Brusca, John S.; Assistant Examiner: Moran, Marjorie A.

LREP Townsend & Townsend and Crew LLP

CLMN Number of Claims: 7

ECL Exemplary Claim: 1

DRWN 0 Drawing Figure(s); 0 Drawing Page(s)

LN.CNT 2792

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to Mycobacterium antigens, optionally from a species such as M. tuberculosis, M. bovis, M. smegmatis, BCG, M. leprae, M. scrofulaceum, M. avium-intracellulare, M. marinum, M. ulcerans, M. kansasii, M. xenopi, M. szulgai, M. fortuitum, or M. chelonae. In particular, the invention relates to M. tuberculosis secretory polypeptides, polynucleotides that encode the polypeptides, and methods of using such compositions in the treatment, prevention and diagnosis of M. tuberculosis infection.

L16 ANSWER 11 OF 65 USPATFULL on STN

AN 2002:209121 USPATFULL

TI Polynucleotide functionally coding for the LHP protein from Mycobacterium tuberculosis, its biologically active derivative fragments, as well as methods using the same

IN Gicquel, Brigitte, Paris, FRANCE

Berthet, Francois-Xavier, Paris, FRANCE

Andersen, Peter, Bronshoj, DENMARK

Rasmussen, Peter Birk, Kobenhavn, DENMARK

PA Institut Pasteur, Paris, FRANCE (non-U.S. corporation)

PI US 6436409 B1 20020820

AI US 1998-116492 19980716 (9)

PRAI US 1997-52631P 19970716 (60)

DT Utility

FS GRANTED

EXNAM Primary Examiner: Swartz, Rodney P

LREP Oblon, Spivak, McClelland, Maier & Neustadt, P.C.

CLMN Number of Claims: 26

ECL Exemplary Claim: 1

DRWN 21 Drawing Figure(s); 17 Drawing Page(s)

LN.CNT 2304

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is directed to a polynucleotide carrying an open reading frame coding for an antigenic polypeptide from Mycobacterium tuberculosis, named lhp, which is placed under the control of its own regulation signals which are functional in mycobacteria, specially in mycobacteria belonging to the Mycobacterium tuberculosis complex and also in fast growing mycobacteria such as Mycobacterium smegmatis. The invention is also directed to the polypeptide LHP encoded by lhp and most preferably to suitable antigenic portions of LHP as well as to oligomeric polypeptides containing more than one unit of LHP or an antigenic portion of LHP. The invention concerns also immunogenic and vaccine compositions containing a polypeptide or an oligomeric polypeptide such as defined above, as well as antibodies directed specifically against such polypeptides that are useful as diagnostic reagents. In another embodiment, the present invention is directed to a polynucleotide carrying the natural regulation signals of lhp which is useful in order to express heterologous proteins in mycobacteria. Finally, the present invention is directed to oligonucleotides

comprising at least 12 consecutive nucleotides from the regulation sequence of lhp which are useful as reagents for detecting the presence of Mycobacterium tuberculosis in a biological sample.

L16 ANSWER 12 OF 65 USPATFULL on STN

AN 2002:50835 USPATFULL

TI Homologously recombinant slow growing mycobacteria and uses therefor

IN Aldovini, Anna, Winchester, MA, United States

Young, Richard A., Winchester, MA, United States

PA Whitehead Institute for Biomedical Research, United States (U.S. corporation)

PI US 6355486 B1 20020312

AI US 1999-342563 19990629 (9)

RLI Continuation of Ser. No. US 1995-471869, filed on 7 Jun 1995, now patented, Pat. No. US 6022745 Continuation of Ser. No. US 1993-95734, filed on 22 Jul 1993, now patented, Pat. No. US 5807723 Continuation-in-part of Ser. No. US 1991-711334, filed on 6 Jun 1991, now abandoned Continuation-in-part of Ser. No. US 1989-367894, filed on 19 Jun 1989, now abandoned Continuation-in-part of Ser. No. US 1989-361944, filed on 5 Jun 1989, now patented, Pat. No. US 5504005 Continuation-in-part of Ser. No. US 1988-223089, filed on 22 Jul 1988, now abandoned Continuation-in-part of Ser. No. US 1988-216390, filed on 7 Jul 1988, now abandoned Continuation-in-part of Ser. No. US 1988-163546, filed on 3 Mar 1988, now abandoned Continuation-in-part of Ser. No. US 1987-20451, filed on 2 Mar 1987, now abandoned

DT Utility

FS GRANTED

EXNAM Primary Examiner: Brusca, John S.

LREP Hamilton, Brook, Smith & Reynolds, P.C.

CLMN Number of Claims: 35

ECL Exemplary Claim: 1

DRWN 5 Drawing Figure(s); 8 Drawing Page(s)

LN.CNT 1516

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method of transforming slow-growing mycobacteria, such as M. bovis BCG, M. leprae, M. tuberculosis, M. avium, M. intracellulare and M. africanum; a method of manipulating genomic DNA of slow-growing mycobacteria through homologous recombination; a method of producing homologously recombinant (HR) slow-growing mycobacteria in which heterologous DNA is integrated into the genomic DNA at a homologous locus; homologously recombinant (HR) slow-growing mycobacteria having heterologous DNA integrated into their genomic DNA at a homologous locus; and mycobacterial DNA useful as a genetic marker.

L16 ANSWER 13 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2002:715772 CAPLUS

DN 138:12597

TI Nitric oxide scavenging and detoxification by the Mycobacterium tuberculosis hemoglobin, HbN in Escherichia coli

AU Pathania, Ranjana; Navani, Naveen K.; Gardner, Anne M.; Gardner, Paul R.; Dikshit, Kanak L.

CS Institute of Microbial Technology, Chandigarh, 160036, India

SO Molecular Microbiology (2002), 45(5), 1303-1314
CODEN: MOMIEE; ISSN: 0950-382X

PB Blackwell Science Ltd.

DT Journal

LA English

AB Nitric oxide (NO), generated in large amts. within macrophages, controls and restricts the growth of internalized human pathogen *Mycobacterium tuberculosis* H37Rv. The mol. mechanism by which tubercle bacilli survive within macrophages is currently of intense interest. In this work, we have demonstrated that dimeric Hb, HbN, from *M. tuberculosis* exhibits distinct nitric oxide dioxygenase (NOD) activity and protects growth and cellular respiration of heterologous hosts, *Escherichia coli* and *Mycobacterium smegmatis*, from the toxic effect of exogenous NO and the NO-releasing compds. A flavoHb (HMP)-deficient mutant of *E. coli*, unable to metabolize NO, acquired an oxygen-dependent NO consumption activity in the presence of HbN. On the basis of cellular heme content, the specific NOD activity of HbN was nearly 35-fold higher than the single-domain *Vitreoscilla* Hb (VHb) but was seven-fold lower than the two-domain flavoHb. HbN-dependent NO consumption was sustained with repeated addn. of NO, demonstrating that HbN is catalytically reduced within *E. coli*. Aerobic growth and respiration of a flavoHb (HMP) mutant of *E. coli* was inhibited in the presence of exogenous NO but remained insensitive to NO inhibition when these cells produced HbN, VHb or flavoHb. *M. smegmatis*, carrying a native HbN very similar to *M. tuberculosis* HbN, exhibited a 7.5-fold increase in NO uptake when exposed to gaseous NO, suggesting NO-induced NOD activity in these cells. In addn., expression of plasmid-encoded HbN of *M. tuberculosis* in *M. smegmatis* resulted in 100-fold higher NO consumption activity than the isogenic control cells. These results provide strong exptl. evidence in support of NO scavenging and detoxification function for the *M. tuberculosis* HbN. The catalytic NO scavenging by HbN may be highly advantageous for the survival of tubercle bacilli during infection and pathogenesis.

RE.CNT 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 14 OF 65 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 3

AN 2001:459107 BIOSIS

DN PREV200100459107

TI Recombinant mycobacterial vaccine.

AU Bloom, Barry R.; Davis, Ronald W.; Jacobs, William R., Jr. (1); Young, Richard A.; Husson, Robert N.

CS (1) Bronx, NY USA

ASSIGNEE: Albert Einstein College of Medicine of Yeshiva University; The Board of Trustees of the Leland Stanford, Jr. University, Palo Alto, CA, USA; Whitehead Institute for Biomedical Research

PI US 6270776 August 07, 2001

SO Official Gazette of the United States Patent and Trademark Office Patents, (Aug. 7, 2001) Vol. 1249, No. 1, pp. No Pagination. e-file.
ISSN: 0098-1133.

DT Patent

LA English

AB Recombinant mycobacterial vaccine vehicles capable of expressing DNA of interest which encodes at least one protein antigen for at least one pathogen against which an immune response is desired and which can be incorporated into the mycobacteria or stably integrated into the mycobacterial genome. The vaccine vehicles are useful for administration to mammalian hosts for purposes of immunization. A recombinant vector which replicates in *E. coli* but not in mycobacteria is also disclosed. The recombinant vector includes 1) a mycobacterial gene or portions thereof, necessary for recombination with homologous sequences in the genome of
mycobacteria ***transformed*** with the recombinant plasmid;

2) all or a portion of a gene which encodes a polypeptide or protein whose expression is desired in ***mycobacteria*** ***transformed*** with the recombinant plasmid; 3) DNA sequences necessary for replication and selection in E. coli; and 4) DNA sequences necessary for selection in mycobacteria (e.g., drug resistance). The present invention also relates to two types of recombinant vectors useful in introducing DNA of interest into mycobacteria, where it is expressed. One type of vector is a recombinant phasmid capable of replicating as a plasmid in E. coli and of lysogenizing a mycobacterial host. The other type of vector is a recombinant plasmid which can be introduced into mycobacteria, where it is stably maintained extrachromosomally.

L16 ANSWER 15 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2001:519386 CAPLUS

DN 135:87989

TI Methods and materials (host cells and vectors) used for identification of genes from uncultivated microorganisms, the gene products of which are involved in biochemical pathways

IN Handelsman, Jo; Goodman, Robert M.; Rondon, Michelle R.

PA Wisconsin Alumni Research Foundation, USA

SO U.S., 32 pp., Cont.-in-part of U.S. Ser. No. 956,692.

CODEN: USXXAM

DT Patent

LA English

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6261842	B1	20010717	US 1997-969651	19971113
	WO 9920799	A2	19990429	WO 1998-US22533	19981023
	WO 9920799	A3	19990805		
	W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
	RW:	GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
	AU 9911193	A1	19990510	AU 1999-11193	19981023
	EP 1023466	A2	20000802	EP 1998-953952	19981023
	R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI			
	JP 2001520055	T2	20011030	JP 2000-517117	19981023
	US 2002045177	A1	20020418	US 2001-877406	20010608
PRAI	US 1997-956692	A2	19971024		
	US 1997-63230P	P	19971023		
	US 1997-969651	A	19971113		
	WO 1998-US22533	W	19981023		

AB The invention provides host cells and genetic vectors which have been engineered to express open reading frames of genomic DNA sub-cloned from a heterologous microorganism, thereby allowing for the identification of genes from uncultivated microbes, the gene products of which are involved in biochem. pathways. The invention specifically provides methods and materials used for prodn. of bacterial DNA and/or soil DNA libraries using the PAC or BAC (pBeloBAC11) cloning vectors, and host cells, such as Escherichia coli or Streptomyces. The invention provides for the use of said libraries for cloning at least one gene of a biosynthetic pathway,

whereby the pathway produces a non-proteinaceous compd. The method was illustrated by the construction of Bacillus cereus DNA and soil DNA libraries using pBeloBAC11. Using this procedure, eight clones from the soil DNA library were shown to degrade esculin, and one clone was shown to have antibacterial activity against Staphylococcus aureus.

RE.CNT 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 16 OF 65 USPATFULL on STN

AN 2001:173322 USPATFULL

TI Mycobacterial species-specific reporter mycobacteriophages

IN Jacobs, Jr., William R., City Island, NY, United States

Bloom, Barry R., Hastings-on-Hudson, NY, United States

Hatfull, Graham F., Pittsburgh, PA, United States

PA Albert Einstein College of Medicine of Yeshiva University, Bronx, NY,

United States (U.S. corporation)

University of Pittsburgh, Pittsburgh, PA, United States (U.S.

corporation)

PI US 6300061 B1 20011009

AI US 1996-705557 19960829 (8)

RLI Continuation of Ser. No. US 1995-430314, filed on 28 Apr 1995, now
abandoned Continuation of Ser. No. US 1993-57531, filed on 29 Apr 1993,
now abandoned Continuation-in-part of Ser. No. US 1992-833431, filed on
7 Feb 1992, now abandoned

DT Utility

FS GRANTED

EXNAM Primary Examiner: Ketter, James

LREP Amster, Rothstein & Ebenstein

CLMN Number of Claims: 8

ECL Exemplary Claim: 1

DRWN 41 Drawing Figure(s); 33 Drawing Page(s)

LN.CNT 2570

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to mycobacterial species-specific reporter mycobacteriophages (reporter mycobacteriophages), methods of producing said reporter mycobacteriophages and the use of said reporter mycobacteriophages for the rapid diagnosis of mycobacterial infection and the assessment of drug susceptibilities of mycobacterial strains in clinical samples. In particular, this invention is directed to the production and use of luciferase reporter mycobacteriophages to diagnose tuberculosis. The mycobacterial species-specific reporter mycobacteriophages comprise mycobacterial species-specific mycobacteriophages which contain reporter genes and transcriptional promoters therein. When the reporter mycobacteriophages are incubated with clinical samples which may contain the mycobacteria of interest, the gene product of the reporter genes will be expressed if the sample contains the mycobacteria of interest, thereby diagnosing mycobacterial infection.

L16 ANSWER 17 OF 65 USPATFULL on STN

AN 2001:121308 USPATFULL

TI IniB, iniA and iniC genes of mycobacteria and methods of use

IN Alland, David, Dobbs Ferry, NY, United States

Bloom, Barry R., Hastings-on-Hudson, NY, United States

Jacobs, Jr., William R., City Island, NY, United States

PA Albert Einstein College of Medicine of Yeshiva University, Bronx, NY,
United States (U.S. corporation)

PI US 6268201 B1 20010731
AI US 1998-177349 19981023 (9)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Swart, Rodney P.
LREP Amster, Rothstein & Ebenstein
CLMN Number of Claims: 4
ECL Exemplary Claim: 1
DRWN 9 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 845

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to the identification, cloning, sequencing and characterization of the iniB, iniA and iniC genes of mycobacteria which are induced by a broad class of antibiotics that act by inhibiting cell wall biosynthesis, including the first line antituberculosis agents, isoniazid and ethambutol. The present invention provides purified and isolated iniB, iniA, iniC and iniB promoter nucleic acids which may comprise the iniBAC operon, as well as mutated forms of these nucleic acids. The present invention also provides one or more single-stranded nucleic acid probes which specifically hybridize to the iniB, iniA, iniC and iniB promoter nucleic acids, and mixtures thereof, which may be formulated in kits, and used in the diagnosis of drug-resistant mycobacterial strain. The present invention also provides methods for the screening and identification of drugs effective against Mycobacterium tuberculosis using induction of the iniB promoter.

L16 ANSWER 18 OF 65 USPATFULL on STN

AN 2001:75171 USPATFULL
TI Recombinant immunogenic actinomycetale
IN Gicquel, Brigitte, Paris, France
Winter, Nathalie, Paris, France
Gheorghiu, Marina, Neuilly-sur-Seine, France
PA Institut Pasteur, Paris, France (non-U.S. corporation)
PI US 6235518 B1 20010522
WO 9325678 19931223
AI US 1994-157152 19940726 (8)
WO 1992-EP1343 19920612
19940726 PCT 371 date
19940726 PCT 102(e) date

PRAI GB 1991-401601 19910614
DT Utility
FS Granted
EXNAM Primary Examiner: Minnifield, Nita
LREP Oblon, Spivak, McClelland, Maier & Neustadt, P.C.
CLMN Number of Claims: 31
ECL Exemplary Claim: 1
DRWN 9 Drawing Figure(s); 6 Drawing Page(s)
LN.CNT 834

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A ***mycobacteria*** ***transformed*** with an antigen-encoding gene, such as nef, under the control of a Streptomyces stress-responsive promoter, such as the S. albus groES/groEL1 promoter, and preferably associated with a synthetic ribosome binding site. The recombinant mycobacteria can be used as a vaccine against, for example, a pathogen which carries the antigen.

L16 ANSWER 19 OF 65 USPATFULL on STN

AN 2001:63444 USPATFULL
 TI Mycobacterial species-specific reporter mycobacteriophages
 IN Jacobs, Jr., William R., City Island, NY, United States
 Bloom, Barry R., Hastings-on-Hudson, NY, United States
 Hatfull, Graham F., Pittsburgh, PA, United States
 PA Albert Einstein College of Medicine of Yeshiva University, Bronx, NY,
 United States (U.S. corporation)
 University of Pittsburgh, Pittsburgh, PA, United States (U.S.
 corporation)
 PI US 6225066 B1 20010501
 AI US 1999-426436 19991025 (9)
 RLI Continuation of Ser. No. US 1996-705557, filed on 29 Aug 1996
 Continuation of Ser. No. US 1995-430314, filed on 28 Apr 1995, now
 abandoned Continuation of Ser. No. US 1993-57531, filed on 29 Apr 1993,
 now abandoned Continuation-in-part of Ser. No. US 1992-833431, filed on
 7 Feb 1992, now abandoned
 DT Utility
 FS Granted
 EXNAM Primary Examiner: Ketter, James
 LREP Amster, Rothstein & Ebenstein
 CLMN Number of Claims: 16
 ECL Exemplary Claim: 1
 DRWN 41 Drawing Figure(s); 33 Drawing Page(s)
 LN.CNT 2581
 CAS INDEXING IS AVAILABLE FOR THIS PATENT.
 AB This invention relates to mycobacterial species-specific reporter
 mycobacteriophages (reporter mycobacteriophages), methods of producing
 said reporter mycobacteriophages and the use of said reporter
 mycobacteriophages for the rapid diagnosis of mycobacterial infection
 and the assessment of drug susceptibilities of mycobacterial strains in
 clinical samples. In particular, this invention is directed to the
 production and use of luciferase reporter mycobacteriophages to diagnose
 tuberculosis. The mycobacterial species-specific reporter
 mycobacteriophages comprise mycobacterial species-specific
 mycobacteriophages which contain reporter genes and transcriptional
 promoters therein. When the reporter mycobacteriophages are incubated
 with clinical samples which may contain the mycobacteria of interest,
 the gene product of the reporter genes will be expressed if the sample
 contains the mycobacteria of interest, thereby diagnosing mycobacterial
 infection.

 L16 ANSWER 20 OF 65 USPATFULL on STN
 AN 2001:10548 USPATFULL
 TI DNA molecule conferring on Mycobacterium tuberculosis resistance against
 antimicrobial reactive oxygen and nitrogen intermediates
 IN Riley, Lee W., Berkeley, CA, United States
 Nathan, Carl F., Larchmont, NY, United States
 Ehrt, Sabine, Berkeley, CA, United States
 PA Cornell Research Foundation, Inc., Ithaca, NY, United States (U.S.
 corporation)
 PI US 6177086 B1 20010123
 AI US 1998-67626 19980428 (9)
 PRAI US 1997-45688P 19970506 (60)
 DT Utility
 FS Granted
 EXNAM Primary Examiner: Swart, Rodney P.
 LREP Nixon Peabody LLP

CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN 31 Drawing Figure(s); 12 Drawing Page(s)
LN.CNT 1844

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a DNA molecule conferring on Mycobacterium tuberculosis resistance to antimicrobial reactive oxygen intermediates and reactive nitrogen intermediates. The protein encoded by this DNA molecule is useful in vaccines to prevent infection by Mycobacterium tuberculosis, while the antibodies raised against this protein can be employed in passively immunizing those already infected by the organism. Both these proteins and antibodies may be utilized in diagnostic assays to detect Mycobacterium tuberculosis in tissue or bodily fluids. The protein or polypeptide is also useful as a therapeutic in treating conditions mediated by the production of reactive oxygen intermediates and nitrogen intermediates.

L16 ANSWER 21 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2001:374302 CAPLUS

DN 136:195098

TI Molecular analysis of Mycobacterium tuberculosis phosphate specific transport system in Mycobacterium smegmatis. Characterization of recombinant 38 kDa (PstS-1)

AU Torres, Ascencion; Juarez, Maria Dolores; Cervantes, Rafael; Espitia, Clara

CS Departamento de Inmunologia, Instituto de Investigaciones Biomedicas, UNAM, Mexico City, Mex.

SO Microbial Pathogenesis (2001), 30(5), 289-297

CODEN: MIPAEV; ISSN: 0882-4010

PB Academic Press

DT Journal

LA English

AB The functionality of the putative Mycobacterium tuberculosis phosphate transport operon was studied by operon- lacZ promoterless fusions in Mycobacterium smegmatis. The expression of the operon genes was evaluated in transformed M. smegmatis growing in medium with low and high phosphate concn. Although the gene fusions expressed .beta.-galactosidase in medium with phosphate, a higher activity was detected in bacteria growing in medium with low phosphate. In contrast, alk. phosphatase activity from M. smegmatis was detected only in bacteria growing in medium with low phosphate. The expression of the operon genes was driven by a promoter located 5' upstream from the start codon of the pstB gene. A second putative internal promoter 5' upstream of the pstS-1 gene was also detected. Furthermore, comparative anal. between the native and recombinant PstS-1 proteins showed that they were very similar. Like the native protein, the recombinant protein was also secreted to the culture medium as a glycosylated band. The results show that M. smegmatis recognized phosphate regulatory signals of the M. tuberculosis phosphate transport operon genes, and open the possibility to study gene phosphate regulation in mycobacteria. (c) 2001 Academic Press.

RE.CNT 55 THERE ARE 55 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 22 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2001:173711 CAPLUS

DN 135:314156

TI Monitoring promoter activity and protein localization in Mycobacterium

spp. using green fluorescent protein

AU Cowley, S. C.; Av-Gay, Y.

CS Division of Infectious Diseases, Department of Medicine, University of British Columbia, Vancouver, BC, V5Z 3J5, Can.

SO Gene (2001), 264(2), 225-231
CODEN: GENED6; ISSN: 0378-1119

PB Elsevier Science B.V.

DT Journal

LA English

AB Two green fluorescent protein (Gfp) fusion vectors were constructed for use in Mycobacterium spp. The first plasmid facilitates quantification of mycobacterial promoter activity. The second vector permits construction of translational fusions of mycobacterial proteins to Gfp in order to study subcellular localization including protein secretion. Using this translational fusion construct, we verify that a Gfp fusion to the putative secreted M. tuberculosis protein ChoD is translocated to the extracellular milieu when cloned and expressed in Mycobacterium smegmatis.

RE.CNT 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 23 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2001:826435 CAPLUS

DN 136:350990

TI Energy transfer between fluorescent proteins using a co-expression system in Mycobacterium smegmatis

AU Kaps, Iris; Ehrt, Sabine; Seeber, Silke; Schnappinger, Dirk; Martin, Carlos; Riley, Lee W.; Niederweis, Michael

CS Lehrstuhl für Mikrobiologie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, D-91058, Germany

SO Gene (2001), 278(1-2), 115-124
CODEN: GENED6; ISSN: 0378-1119

PB Elsevier Science B.V.

DT Journal

LA English

AB The goal of this study was to establish a two-plasmid co-expression system for Mycobacterium smegmatis. Two vectors with compatible origins of replication and a polylinker, which allows modular cloning of promoters and genes, were constructed and used to clone genes encoding a blue fluorescent protein (BFP) and a green fluorescent protein (GFP). A 160-fold variation of GFP expression levels in M. smegmatis was achieved by combining three promoters with different copy nos. of the vectors. An efficient energy transfer between BFP and GFP in M. smegmatis was obsd. by fluorescence measurements and demonstrated that these genes were simultaneously expressed from both vectors. Thus, these vectors will be valuable for all strategies where co-expression of proteins in M. smegmatis is needed, e.g. for constructing a two-hybrid system or for deleting essential genes.

RE.CNT 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 24 OF 65 LIFESCI COPYRIGHT 2003 CSA on STN

AN 2001:89014 LIFESCI

TI Recombinant mycobacterial vaccine

AU Bloom, B.R.; Davis, R.W.; Jacobs, W.R..Jr.; Young, R.A.; Husson, R.N.

CS Albert Einstein College of Medicine of Yeshiva University

SO (20010807) . US Patent: 6270776; US CLASS: 424/248.1; 435/69.1; 435/69.3; 435/455; 435/71.1; 435/253.1; 424/93.1.

DT Patent
FS W3
LA English
SL English
AB Recombinant mycobacterial vaccine vehicles capable of expressing DNA of interest which encodes at least one protein antigen for at least one pathogen against which an immune response is desired and which can be incorporated into the mycobacteria or stably integrated into the mycobacterial genome. The vaccine vehicles are useful for administration to mammalian hosts for purposes of immunization. A recombinant vector which replicates in E. coli but not in mycobacteria is also disclosed. The recombinant vector includes 1) a mycobacterial gene or portions thereof, necessary for recombination with homologous sequences in the genome of ***mycobacteria*** ***transformed*** with the recombinant plasmid; 2) all or a portion of a gene which encodes a polypeptide or protein whose expression is desired in ***mycobacteria*** ***transformed*** with the recombinant plasmid; 3) DNA sequences necessary for replication and selection in E. coli; and 4) DNA sequences necessary for selection in mycobacteria (e.g., drug resistance). The present invention also relates to two types of recombinant vectors useful in introducing DNA of interest into mycobacteria, where it is expressed. One type of vector is a recombinant phasmid capable of replicating as a plasmid in E. coli and of lysogenizing a mycobacterial host. The other type of vector is a recombinant plasmid which can be introduced into mycobacteria, where it is stably maintained extrachromosomally.

L16 ANSWER 25 OF 65 USPATFULL on STN

AN 2000:74132 USPATFULL

TI Shuttle vectors for the introduction of DNA into mycobacteria and utilization of such bacteria as vaccines

IN Escuyer, Vincent, Massy, France

Baulard, Alain, Tournai, France

Berche, Patrick, Saint-Cloud, France

Locht, Camille, Wannehain, France

Haddad, Nadia, Paris, France

PA Institute National de la Sante et de la Recherche Medical (Inserm), Paris Cedex, France (non-U.S. corporation)

Institut Pasteur de Lille, Lille Cedex, France (non-U.S. corporation)

PI US 6074866 20000613

WO 9532296 19951130

AI US 1997-737588 19970212 (8)

WO 1995-FR664 19950519

19970212 PCT 371 date

19970212 PCT 102(e) date

PRAI FR 1994-6202 19940520

DT Utility

FS Granted

EXNAM Primary Examiner: Yucel, Remy

LREP Greenblum & Berstein, P.L.C.

CLMN Number of Claims: 7

ECL Exemplary Claim: 1

DRWN 9 Drawing Figure(s); 9 Drawing Page(s)

LN.CNT 559

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Shuttle vectors for inserting DNA in mycobacteria comprising at least one origin of functional replication in said mycobacteria, another origin of functional replication in other bacteria, an enzyme cutting

site allowing the insertion of DNA coding for a protein capable of being expressed in the mycobacteria, characterized in that they also carry a gene providing on said mycobacteria resistance to a compound containing a heavy metal.

L16 ANSWER 26 OF 65 USPATFULL on STN

AN 2000:15521 USPATFULL

TI Homologously recombinant slow growing mycobacteria and uses therefor

IN Aldovini, Anna, Weston, MA, United States

Young, Richard A., Weston, MA, United States

PA Whitehead Institute for Biomedical Research, Cambridge, MA, United States (U.S. corporation)

PI US 6022745 20000208

AI US 1995-471869 19950607 (8)

RLI Continuation of Ser. No. US 1993-95734, filed on 22 Jul 1993, now patented, Pat. No. US 5807723 which is a continuation-in-part of Ser. No. US 1991-711334, filed on 6 Jun 1991, now abandoned which is a continuation-in-part of Ser. No. US 1989-367894, filed on 19 Jun 1989, now abandoned which is a continuation-in-part of Ser. No. US 1989-361944, filed on 5 Jun 1989, now patented, Pat. No. US 5504005 which is a continuation-in-part of Ser. No. US 1988-223089, filed on 22 Jul 1988, now abandoned And Ser. No. US 1988-216390, filed on 7 Jul 1988, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Brusca, John S.

LREP Hamilton, Brook, Smith & Reynolds, P.C.

CLMN Number of Claims: 29

ECL Exemplary Claim: 1

DRWN 8 Drawing Figure(s); 7 Drawing Page(s)

LN.CNT 1472

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method of transforming slow-growing mycobacteria, such as *M. bovis* BCG, *M. leprae*, *M. tuberculosis* *M. avium*, *M. intracellulare* and *M. africanum*; a method of manipulating genomic DNA of slow-growing mycobacteria through homologous recombination; a method of producing homologously recombinant (HR) slow-growing mycobacteria in which heterologous DNA is integrated into the genomic DNA at a homologous locus; homologously recombinant (HR) slow-growing mycobacteria having heterologous DNA integrated into their genomic DNA at a homologous locus; and mycobacterial DNA useful as a genetic marker.

L16 ANSWER 27 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2000:620032 CAPLUS

DN 134:142473

TI Recombinant BCG approach for development of vaccines: cloning and expression of immunodominant antigens of *M. tuberculosis*

AU Dhar, N.; Rao, V.; Tyagi, A. K.

CS Department of Biochemistry, University of Delhi South Campus, New Delhi, 110021, India

SO FEMS Microbiology Letters (2000), 190(2), 309-316

CODEN: FMLED7; ISSN: 0378-1097

PB Elsevier Science B.V.

DT Journal

LA English

AB In spite of major advances in our understanding of the biol. and immunol. of tuberculosis, the incidence of the disease has not reduced in most

parts of the world. In an attempt to improve the protective efficacy of *Mycobacterium bovis* bacille Calmette-Guerin (BCG), we have developed a generic vector system, pSD5, for expression of genes at varying levels in mycobacteria. In this study, we have cloned and overexpressed three immunodominant secretory antigens of *M. tuberculosis*, 85A, 85B and 85C, belonging to the antigen 85 complex. All the genes were cloned under the control of a battery of mycobacterial promoters of varying strength. The expression was analyzed in the fast-growing strain *M. smegmatis* and the slow-growing vaccine strain *M. bovis* BCG. The recombinant BCG constructs were able to express the antigens at high levels and the majority of the expressed antigens was secreted into the medium. These results show that by using this strategy the recombinant BCG approach can be successfully used for the development of candidate vaccines against infections associated with mycobacteria as well as other pathogens.

RE.CNT 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 28 OF 65 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 4

AN 2000:118645 BIOSIS

DN PREV200000118645

TI Transformation and transposition of the genome of *Mycobacterium marinum*.

AU Talaat, Adel M. (1); Trucksis, Michele

CS (1) University of Texas Southwestern Medical Center, 5323 Harry Hines
Blvd, Dallas, TX, 75235 USA

SO American Journal of Veterinary Research, (Feb., 2000) Vol. 61, No. 2, pp.
125-128.

ISSN: 0002-9645.

DT Article

LA English

SL English

AB Objective: To develop and evaluate protocols for genetic manipulations (transformation and transposition) of the fish pathogen, *Mycobacterium marinum*. Sample Population: Isolates of *M. marinum* obtained from fish and humans. Procedure: Electrocompetent cells were prepared from isolates of *M. marinum* grown to various growth phases at several temperatures and with or without the addition of ethionamide or cycloheximide.
Mycobacterial cells were ***transformed*** by electroporation with a replicative *Escherichia coli*-mycobacteria shuttle vector (pYUB18) as well as suicide vectors (pYUB285 and pUS252) that carried transposable elements (IS 1096 and IS 6110, respectively). Mutants from both isolates of *M. marinum* were recovered on 7H10 agar plates supplemented with kanamycin. Transformation and transposition efficiencies for various protocols were compared. Southern hybridization analysis was performed on mycobacterial mutants to confirm transposition events. Results: Competent cells prepared at room temperature (23-25 C) from organisms in late-exponential growth phase yielded higher transposition efficiency, compared with cells prepared at 4 C or from organisms in early- or mid-exponential growth phase. Naturally developing kanamycin-resistant colonies of *M. marinum* were not detected. Only the IS 1096-derived transposition was able to efficiently mutate *M. marinum*. Southern hybridization of *M. marinum* mutants revealed random integration of IS 1096 into the *M. marinum* genome. Conclusions: Transposition and transformation efficiencies were comparable, suggesting that the limiting factor in transposition is the transformation step. Most of the experiments resulted in transposition of IS 1096; however, better approaches are needed to improve transposition efficiency.

L16 ANSWER 29 OF 65 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 5
 AN 2000:289813 BIOSIS
 DN PREV2000000289813
 TI Mycobacteriophages and uses thereof.
 AU Bloom, Barry R. (1); Davis, Ronald W.; Jacobs, William R.; Young, Richard
 A.; Husson, Robert N.
 CS (1) Takoma Park, MD USA
 ASSIGNEE: Albert Einstein College of Medicine of Yeshiva University,
 Bronx, NY, USA; Albert Einstein College of Medicine of Yeshiva University,
 Bronx, NY, USA; Whitehead Institute for Biomedical Research, Cambridge,
 MA, USA
 PI US 5968733 October 19, 1999
 SO Official Gazette of the United States Patent and Trademark Office Patents,
 (Oct. 19, 1999) Vol. 1227, No. 3, pp. No pagination. e-file.
 ISSN: 0098-1133.
 DT Patent
 LA English
 AB Recombinant mycobacterial vaccine vehicles capable of expressing DNA of
 interest which encodes at least one protein antigen for at least one
 pathogen against which an immune response is desired and which can be
 incorporated into the mycobacteria or stably integrated into the
 mycobacterial genome. The vaccine vehicles are useful for administration
 to mammalian hosts for purposes of immunization. A recombinant vector
 which replicates in E. coli but not in mycobacteria is also disclosed. The
 recombinant vector includes 1) a mycobacterial gene or portions thereof,
 necessary for recombination with homologous sequences in the genome of
 mycobacteria ***transformed*** with the recombinant plasmid;
 2) all or a portion of a gene which encodes a polypeptide or protein whose
 expression is desired in ***mycobacteria*** ***transformed*** with
 the recombinant plasmid; 3) DNA sequences necessary for replication and
 selection in E. coli; and 4) DNA sequences necessary for selection in
 mycobacteria (e.g., drug resistance). The present invention also relates
 to two types of recombinant vectors useful in introducing DNA of interest
 into mycobacteria, where it is expressed. One type of vector is a
 recombinant phasmid capable of replicating as a plasmid in E. coli and of
 lysogenizing a mycobacterial host. The other type of vector is a
 recombinant plasmid which can be introduced into mycobacteria, where it is
 stably maintained extrachromosomally.

L16 ANSWER 30 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1999:468593 CAPLUS
 DN 131:101258
 TI Materials and methods for treating oncological disease
 IN Lawman, Patricia; Lawman, Michael J. P.
 PA Morphogenesis, Inc., USA
 SO PCT Int. Appl., 37 pp.
 CODEN: PIXXD2
 DT Patent
 LA English
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	-----	----	-----	-----	-----
PI	WO 9936433	A2	19990722	WO 1999-US787	19990114
	WO 9936433	A3	19990923		
	W: CA, JP, US				

RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
PT, SE

US 2002141981 A1 20021003 US 2001-950374 20010910
PRAI US 1998-71497P P 19980114
WO 1999-US787 A1 19990114
US 1999-394226 B1 19990913

AB Novel methods are disclosed for treating oncol. disorders in an individual or animal using a superantigen expressed in tumor cells. A gene encoding a superantigen, such as an M-like protein of group A streptococci, can be introduced into a tumor cell in order to make the tumor cell more immunogenic in the host. Also contemplated are methods wherein a cell expresses a superantigen or superantigens, and immunogenic or immunostimulatory proteins, such as foreign MHC, cytokines, porcine-derived hyperacute rejection antigen, Mycobacterium-derived antigens, and the like. The subject invention also pertains to cells transformed with polynucleotides encoding a superantigen and foreign MHC antigen, cytokines, and other immunogenic or immunostimulatory proteins. Transformed cells according to the subject invention are then provided to an individual or animal in need of treatment for an oncol. disorder. The immune response to tumor cells transformed according to the present invention inhibits in vivo tumor growth and results in subsequent tumor regression. The subject invention also pertains to cell lines transformed with genes encoding a superantigen and, optionally, a foreign Class II MHC antigen and/or a cytokine.

L16 ANSWER 31 OF 65 USPATFULL on STN

AN 1999:72253 USPATFULL

TI Recombinant polypeptides and peptides, nucleic acids coding for the same and use of these polypeptides and peptides in the diagnostic of tuberculosis

IN Content, Jean, Rhode St Genese, Belgium

De Wit, Lucas, Puurs, Belgium

De Bruyn, Jacqueline, Beersel, Belgium

Van Vooren, Jean-Paul, St-Pieters Leeuw, Belgium

PA N.V. Innogenetics S.A., Ghent, Belgium (non-U.S. corporation)

PI US 5916558 19990629

AI US 1995-447430 19950522 (8)

RLI Continuation of Ser. No. US 690949

PRAI GB 1989-402571 19890919

DT Utility

FS Granted

EXNAM Primary Examiner: Marschel, Ardin H.

LREP Fish & Richardson P.C.

CLMN Number of Claims: 44

ECL Exemplary Claim: 1

DRWN 62 Drawing Figure(s); 60 Drawing Page(s)

LN.CNT 5009

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to recombinant polypeptides and peptides which can also be used for the diagnosis of tuberculosis. The invention also relates to a process for preparing the above polypeptides and peptides, which are in a state of biological purity such that they can be used as part of the active principle in the preparation of vaccines against tuberculosis. The invention additionally relates to nucleic acids coding for said polypeptides and peptides.

L16 ANSWER 32 OF 65 USPATFULL on STN

AN 1999:15759 USPATFULL
TI Homologously recombinant slow growing mycobacteria and uses therefor
IN Aldovini, Anna, Winchester, MA, United States
Young, Richard A., Winchester, MA, United States
PA Whitehead Institute for Biomedical Research, Cambridge, MA, United States (U.S. corporation)
PI US 5866403 19990202
AI US 1995-444623 19950519 (8)
RLI Division of Ser. No. US 1993-95734, filed on 22 Jul 1993 which is a continuation-in-part of Ser. No. US 1991-711334, filed on 6 Jun 1991, now abandoned which is a continuation-in-part of Ser. No. US 1989-367894, filed on 19 Jun 1989, now abandoned which is a continuation-in-part of Ser. No. US 1989-361944, filed on 5 Jun 1989, now patented, Pat. No. US 5504005 which is a continuation-in-part of Ser. No. US 1988-223089, filed on 22 Jul 1988, now abandoned And Ser. No. US 1988-216390, filed on 14 Jun 1988, now patented, Pat. No. US 4816708 , each Ser. No. US which is a continuation-in-part of Ser. No. US 1988-163546, filed on 3 Mar 1988, now abandoned And Ser. No. US 1987-20451, filed on 2 Mar 1987, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Degen, Nancy; Assistant Examiner: Brusca, John S.
LREP Hamilton, Brook, Smith & Reynolds, P.C.
CLMN Number of Claims: 36
ECL Exemplary Claim: 2
DRWN 8 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 1318

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method of transforming slow-growing mycobacteria, such as *M. bovis* BCG, *M. leprae*, *M. tuberculosis* *M. avium*, *M. intracellulare* and *M. africanum*; a method of manipulating genomic DNA of slow-growing mycobacteria through homologous recombination; a method of producing homologously recombinant (HR) slow-growing mycobacteria in which heterologous DNA is integrated into the genomic DNA at a homologous locus; homologously recombinant (HR) slow-growing mycobacteria having heterologous DNA integrated into their genomic DNA at a homologous locus; and mycobacterial DNA useful as a genetic marker.

L16 ANSWER 33 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1999:592404 CAPLUS

DN 131:347372

TI Stimulation of transposition of the *Mycobacterium tuberculosis* insertion sequence IS6110 by exposure to a microaerobic environment

AU Ghanekar, Kiran; McBride, Alan; Dellagostin, Odir; Thorne, Stephen; Mooney, Rachel; McFadden, Johnjoe

CS Molecular Microbiology Group, School of Biological Sciences, University of Surrey, Surrey, GU2 5XH, UK

SO Molecular Microbiology (1999), 33(5), 982-993

CODEN: MOMIEE; ISSN: 0950-382X

PB Blackwell Science Ltd.

DT Journal

LA English

AB The *Mycobacterium tuberculosis*-specific insertion sequence IS6110/986 has been widely used as a probe because of the multiple polymorphism obsd. among different strains. To investigate transposition of IS6110, a series of artificially constructed composite transposons contg. IS6110 and a kanamycin resistance marker were constructed. The composite transposons

were inserted into a conditionally replicating, thermosensitive, *Escherichia coli*-mycobacterial shuttle vector and introduced into *M. smegmatis* mc2155. Lawns of transformants were grown at the permissive temp. on kanamycin-supplemented agar and subsequently prevented from further growth by shifting to the non-permissive temp. Under normal atm. conditions, kanamycin-resistant papillae appeared after only about 5-6 wk of incubation. However, these events were not assocd. with transposon mobilization. In contrast, lawns that were exposed to a 48h microaerobic shock generated kanamycin-resistant papillae after only 6-14 days. These events were generated by conservative transposition of the IS6110 composite transposon into the *M. smegmatis* chromosome, with loss of the shuttle vector. In common with other IS3 family elements, transposition of IS6110 is thought to be controlled by translational frameshifting. However, we were unable to detect any significant frameshifting within the putative frameshifting site of IS6110, and the level of frameshifting was not affected by microaerobic incubation. The finding that transposition of IS6110 is stimulated by incubation at reduced oxygen tensions may be relevant to transposition of IS6110 in *M. tuberculosis* harbored within TB lesions.

RE.CNT 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 34 OF 65 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 6
AN 1999:118513 BIOSIS
DN PREV199900118513
TI Identification of promoter elements in mycobacteria: Mutational analysis
of a highly symmetric dual promoter directing the expression of
replication genes of the *Mycobacterium* plasmid pAL5000.
AU Stolt, Pelle,; Zhang, Qiuwang (1); Ehlers, Stefan
CS (1) Div. Mol. Infect. Biol., Res. Cent. Borstel, Parkallee 22, D-23845
Borstel Germany
SO Nucleic Acids Research, (Jan. 15, 1999) Vol. 27, No. 2, pp. 396-402.
ISSN: 0305-1048.
DT Article
LA English
AB The 120 bp origin of replication (ori) for the *Mycobacterium* plasmid
pAL5000 has been shown to comprise the binding sites for the replication
protein RepB as well as the start site of transcription for the repA and
repB genes, encoding the replication proteins RepA and RepB. In this work
it is demonstrated that a third gene product, Rap, is involved in
replication in addition to the previously described proteins.

****Mycobacterium**** *smegmatis* cells ***transformed*** with
replicons

carrying the rap gene recover markedly faster upon electroporation than
those transformed with the minimal replicon, which lacks rap. The rap
gene, oppositely orientated to repA/B, was shown to be transcribed from a
promoter orientated back-to-back to and overlapping the repA/B promoter.
As a consequence of the extensive dyad symmetry in this region the two
promoters share several elements, most of which are situated inside the
high-affinity RepB-binding motif in the ori. Transcription of rap runs
through the low-affinity RepB-binding site, which is part of the ori and
necessary for replication. Both promoters were shown to be repressed by
RepB. These divergent promoters were studied through site-specific
mutagenesis in a xylE reporter gene assay. The analysis furnished evidence
supporting the existence of a distal as well as a proximal element in
mycobacterial promoters.

L16 ANSWER 35 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1999:740763 CAPLUS

DN 132:232499

TI The construction of Schistosoma japonicum vaccine BCG-Sj26GST and its identification

AU Huangfu, Yongmu; Zheng, Bo; Cheng, Jizhong; Liang, Juqing; Feng, Zuohua

CS Department of Medical Molecular Biology, Tongji Medical University, Wuhan, 430030, Peop. Rep. China

SO Journal of Tongji Medical University (1999), 19(3), 161-165

CODEN: JTMUEI; ISSN: 0257-716X

PB Tongji Medical University

DT Journal

LA English

AB The expression of foreign gene, Schistosoma japonicum 26 kilodalton antigen (Sj26GST, 26-kilodalton glutathione S-transferase), in Bacillus Calmette-Guerin (BCG), Mycobacterium (M. smegmatis) and Escherichia coli (E. coli) were studied. The cDNA fragment encoding Sj26GST was amplified by PCR using plasmid pGEX, which could express Sj26GST in E. coli as template. The Sj26GST cDNA was cloned into the downstream of human M. tuberculosis heat shock protein (hsp) 70 promoter with correct reading frame, and then the DNA fragment contg. hsp70 promoter and Sj26GST gene were subcloned together into E. coli-Mycobacteria shuttle plasmid pBCG-2000 to construct the expression shuttle plasmid pBCG-Sj26. The recombinant BCG and M. smegmatis mc2155, which were electroplated with pBCG-Sj26, could express Sj26GST and the recombinant S. japonicum vaccine BCG-Sj26GST was made. The recombinant Sj26GST (rSj26GST) were sol. and could be obsd. on SDS-PAGE at mol. wt. of 26-kilodalton. The content of rSj26GST accounted for 15% and 10% of total bacterial protein in BCG and M. smegmatis resp. The results of Western blot showed the combination of rSj26GST with antibody of GST.

RE.CNT 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 36 OF 65 LIFESCI COPYRIGHT 2003 CSA on STN

AN 2000:112545 LIFESCI

TI Mycobacteriophages and uses thereof

AU Bloom, B.; Davis, R.; Jacobs, Jr., W.; Young, R.; Husson, R.

CS Albert Einstein College of Medicine of Yeshiva University

SO (19991019) . US Patent: 5968733; US CLASS: 435/5; 435/6; 435/252.3; 435/320.1; 435/440; 435/471; 536/23.1..

DT Patent

FS W3

LA English

SL English

AB Recombinant mycobacterial vaccine vehicles capable of expressing DNA of interest which encodes at least one protein antigen for at least one pathogen against which an immune response is desired and which can be incorporated into the mycobacteria or stably integrated into the mycobacterial genome. The vaccine vehicles are useful for administration to mammalian hosts for purposes of immunization. A recombinant vector which replicates in E. coli but not in mycobacteria is also disclosed. The recombinant vector includes 1) a mycobacterial gene or portions thereof, necessary for recombination with homologous sequences in the genome of ***mycobacteria*** ***transformed*** with the recombinant plasmid; 2) all or a portion of a gene which encodes a polypeptide or protein whose expression is desired in ***mycobacteria*** ***transformed*** with

the recombinant plasmid; 3) DNA sequences necessary for replication and selection in E. coli; and 4) DNA sequences necessary for selection in mycobacteria (e.g., drug resistance). The present invention also relates to two types of recombinant vectors useful in introducing DNA of interest into mycobacteria, where it is expressed. One type of vector is a recombinant phasmid capable of replicating as a plasmid in E. coli and of lysogenizing a mycobacterial host. The other type of vector is a recombinant plasmid which can be introduced into mycobacteria, where it is stably maintained extrachromosomally.

L16 ANSWER 37 OF 65 JAPIO (C) 2003 JPO on STN
 AN 1998-094397 JAPIO
 TI SELECTION OF INTERALLELIC RECOMBINATION MUTANT
 IN PELICIC VLADIMIR; REYRAT JEAN-MARC; GICQUEL BRIGITTE; GUILHOT CHRISTOPHE;
 JACKSON MARY
 PA INST PASTEUR
 PI JP 10094397 A 19980414 Heisei
 AI JP 1997-190376 (JP09190376 Heisei) 19970611
 PRAI US 1996-661658 19960611
 SO PATENT ABSTRACTS OF JAPAN (CD-ROM), Unexamined Applications, Vol. 1998
 AB PROBLEM TO BE SOLVED: To select the subject mutant by transforming a Mycobacterium strain with a vector including the Sac B gene coding levan saccharase and the objective base sequence and proliferating the strain in a sucrose-containing culture medium.
 SOLUTION: In the process for substitution of the base sequence of the genome of Mycobacterium strain, a vector is prepared so that it may contain the Sac B gene coding levan saccharase and the objective base sequence and the ***Mycobacterium*** strain is ***transformed*** with the vecotor. Then, the clone of the transformed Mycobacterium is proliferated in a sucrose-containing culture medium to select the transformed clone and the recombinant strain is isolated. Thus, the objective interallelic recombination mutant is selected by the method useful for the positive selection of interallelic recombination mutant in the tubercule bacillus complex.
 COPYRIGHT: (C)1998,JPO

L16 ANSWER 38 OF 65 USPATFULL on STN
 AN 1998:162325 USPATFULL
 TI Recombinant mycobacteria
 IN Bloom, Barry R., Hastings on Hudson, NY, United States
 Jacobs, Jr., William R., Bronx, NY, United States
 Davis, Ronald W., Palo Alto, CA, United States
 Young, Richard A., Winchester, MA, United States
 Husson, Robert N., Takoma Park, MD, United States
 PA Albert Einstein College of Medicine of Yeshiva University, a Division of Yeshiva University, Bronx, NY, United States (U.S. corporation)
 PI US 5854055 19981229
 AI US 1995-463942 19950605 (8)
 RLI Continuation of Ser. No. US 1989-361944, filed on 5 Jun 1989, now patented, Pat. No. US 5504005 which is a continuation-in-part of Ser. No. US 1988-223089, filed on 22 Jul 1988, now abandoned And Ser. No. US 1988-216390, filed on 7 Jul 1988, now abandoned , said Ser. No. US -361944 Ser. No. Ser. No. US -223089 And Ser. No. US -216390 which is a continuation-in-part of Ser. No. US 1988-163546, filed on 3 Mar 1988, now abandoned which is a continuation-in-part of Ser. No. US 1987-20451, filed on 2 Mar 1987, now abandoned
 DT Utility

- b) transfecting the mycobacterium strain with the vector;
- c) selecting clones of the resulting transfected mycobacteria for replacement of the nucleotide sequence of interest by propagating the transfected clones in a culture medium supplemented with sucrose; and
- d) isolating the recombinant strain.

The process is useful for positive selection of allelic exchange mutants, such as in *Mycobacterium tuberculosis* complex.

L16 ANSWER 40 OF 65 USPATFULL on STN

AN 1998:128137 USPATFULL

TI Regulation of a sigma factor from *Mycobacterium tuberculosis*

IN Bishai, William R., Baltimore, MD, United States

DeMaio, James, Tacoma, WA, United States

PA The Johns Hopkins University, Baltimore, MD, United States (U.S. corporation)

PI US 5824546 19981020

AI US 1996-622352 19960327 (8)

DT Utility

FS Granted

EXNAM Primary Examiner: Elliott, George C.; Assistant Examiner: McKelvey, Terry A.

LREP Cushman Darby & Cushman IP Group of Pillsbury Madison & Sutro LLP

CLMN Number of Claims: 10

ECL Exemplary Claim: 1

DRWN 6 Drawing Figure(s); 5 Drawing Page(s)

LN.CNT 1139

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Two genes, *orfX* and *orfY*, regulate *sigF* expression and *sigF* activity in *M. tuberculosis*. *M. tuberculosis sigF*, *orfX*, and *orfY* are used in screening methods for potential therapeutic agents which regulate the growth of *M. tuberculosis*.

L16 ANSWER 41 OF 65 USPATFULL on STN

AN 1998:111808 USPATFULL

TI Homologously recombinant slow growing mycobacteria and uses therefor

IN Aldovini, Anna, Winchester, MA, United States

Young, Richard A., Winchester, MA, United States

PA Whitehead Institute for Biomedical Research, Cambridge, MA, United States (U.S. corporation)

PI US 5807723 19980915

AI US 1993-95734 19930722 (8)

RLI Continuation-in-part of Ser. No. US 1991-711334, filed on 6 Jun 1991, now abandoned which is a continuation-in-part of Ser. No. US 1989-367894, filed on 19 Jun 1989, now abandoned And a continuation-in-part of Ser. No. US 1989-361944, filed on 5 Jun 1989, now patented, Pat. No. US 5504005 Ser. No. Ser. No. US 1988-223089, filed on 22 Jul 1988, now abandoned Ser. No. Ser. No. US 1988-216390, filed on 7 Jul 1988, now abandoned Ser. No. Ser. No. US 1988-163546, filed on 3 Mar 1988, now abandoned Ser. No. Ser. No. US 1987-20451, filed on 2 Mar 1987, now abandoned And Ser. No. US 1993-96027, filed on 22 Jul 1993, now patented, Pat. No. US 5591632

DT Utility

FS Granted

EXNAM Primary Examiner: Ketter, James; Assistant Examiner: Brusca, John S.

LREP Hamilton, Brook, Smith & Reynolds, P.C.
CLMN Number of Claims: 28
ECL Exemplary Claim: 1
DRWN 8 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 1337

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method of transforming slow-growing mycobacteria, such as *M. bovis* BCG, *M. leprae*, *M. tuberculosis* *M. avium*, *M. intracellulare* and *M. africanum*; a method of manipulating genomic DNA of slow-growing mycobacteria through homologous recombination; a method of producing homologously recombinant (HR) slow-growing mycobacteria in which heterologous DNA is integrated into the genomic DNA at a homologous locus; homologously recombinant (HR) slow-growing mycobacteria having heterologous DNA integrated into their genomic DNA at a homologous locus; and mycobacterial DNA useful as a genetic marker.

L16 ANSWER 42 OF 65 USPATFULL on STN

AN 1998:36577 USPATFULL

TI Vectors and prokaryotes which autocatalytically delete antibiotic resistance

IN Haun, Shirley L., Gaithersburg, MD, United States
Stover, Charles K., Mercer Island, WA, United States
Hatfull, Graham, Pittsburgh, PA, United States
Hanson, Mark S., Columbia, MD, United States
Jacobs, William R., City Island, NY, United States

PA MedImmune, Inc., Gaithersburg, MD, United States (U.S. corporation)

PI US 5736367 19980407

AI US 1995-425380 19950420 (8)

RLI Continuation-in-part of Ser. No. US 1992-861002, filed on 31 Mar 1992

DT Utility

FS Granted

EXNAM Primary Examiner: Fleisher, Mindy; Assistant Examiner: Weiss, Bonnie D.

LREP Herron, Charles J., Olstein, Elliot M.

CLMN Number of Claims: 14

ECL Exemplary Claim: 1

DRWN 42 Drawing Figure(s); 39 Drawing Page(s)

LN.CNT 1027

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A vector and a prokaryote transformed therewith which includes nucleic acid sequences which make possible the autocatalytic deletion of nucleotide sequences encoding an antibiotic resistance phenotype. The prokaryote can be a bacterium, and in particular a ***mycobacterium***. Such ***transformed*** mycobacteria may be employed in vaccines, thereby eliminating the attendant risk of vaccines including antibiotic resistance markers.

L16 ANSWER 43 OF 65 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 7

AN 1999:8557 BIOSIS

DN PREV199900008557

TI Analysis of the *Mycobacterium bovis* hsp60 promoter activity in recombinant *Mycobacterium avium*.

AU Batoni, Giovanna (1); Maisetta, Giuseppantonio; Florio, Walter; Freer, Giulia; Campa, Mario; Senesi, Sonia

CS (1) Dip. Patol. Seprimentale Biotechnol. Med. Infettivol. Epidemiol., Univ. Pisa, Via S. Zeno 35/39, 56127 Pisa Italy

SO FEMS Microbiology Letters, (Dec. 1, 1998) Vol. 169, No. 1, pp. 117-124.

ISSN: 0378-1097.

DT Article

LA English

AB A clinical isolate of ***Mycobacterium*** avium was
transformed with a new shuttle plasmid containing the Escherichia coli betagalactosidase reporter gene under the control of the Mycobacterium bovis bacillus Calmette-Guerin (BCG) hsp60 promoter. betaGalactosidase activity was assayed spectrophotometrically in bacterial homogenates of the recombinant strain (M. avium::lacZ) and used for quantification of the hsp60 promoter strength in different conditions of extra- and intracellular growth. Very low levels of beta-galactosidase were recorded during the exponential phase of in vitro growth, while they increased progressively during the late exponential and stationary phases. A significant increase in enzyme activity was also induced in exponentially growing cells by shifting the incubation temperature from 37 to 45degree C, but not from 37 to 42degree C nor from 30 to 42degree C. No induction of the promoter was observed by adding hydrogen peroxide to the cultures. Finally, beta-galactosidase levels were quantified during growth of M. avium::lacZ in murine macrophages. Soon after phagocytosis and, to a lesser extent at 1, 5 and 7 days after infection, increased levels of bacterial beta-galactosidase were observed indicating an increment in transcriptional activity of hsp60 promoter both at early phases of infection and during the course of intracellular growth.

L16 ANSWER 44 OF 65 LIFESCI COPYRIGHT 2003 CSA on STN

AN 2000:77417 LIFESCI

TI Recombinant mycobacteria

AU Bloom, B.; Jacobs, Jr., W.; Davis, R.; Young, R.; Husson, R.

CS Albert Einstein College of Medicine of Yeshiva University, a Division of
SO (19981229) . US Patent: 5854055; US CLASS: 435/253.1; 435/69.1; 435/69.8;
435/71.1; 435/172.1; 435/172.3; 435/252.3..

DT Patent

FS W3

LA English

SL English

AB Recombinant mycobacterial vaccine vehicles capable of expressing DNA of interest which encodes at least one protein antigen for at least one pathogen against which an immune response is desired and which can be incorporated into the mycobacteria or stably integrated into the mycobacterial genome. The vaccine vehicles are useful for administration to mammalian hosts for purposes of immunization. A recombinant vector which replicates in E. coli but not in mycobacteria is also disclosed. The recombinant vector includes 1) a mycobacterial gene or portions thereof, necessary for recombination with homologous sequences in the genome of ***mycobacteria*** ***transformed*** with the recombinant plasmid; 2) all or a portion of a gene which encodes a polypeptide or protein whose expression is desired in ***mycobacteria*** ***transformed*** with the recombinant plasmid; 3) DNA sequences necessary for replication and selection in E. coli; and 4) DNA sequences necessary for selection in mycobacteria (e.g., drug resistance). The present invention also relates to two types of recombinant vectors useful in introducing DNA of interest into mycobacteria, where it is expressed. One type of vector is a recombinant plasmid capable of replicating as a plasmid in E. coli and of lysogenizing a mycobacterial host. The other type of vector is a recombinant plasmid which can be introduced into mycobacteria, where it is stably maintained extrachromosomally.

L16 ANSWER 45 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1997:256505 CAPLUS
DN 126:327955
TI Contribution of .beta.-lactamase production to the resistance of mycobacteria to .beta.-lactam antibiotics
AU Quinting, Birgit; Reyrat, Jean-Marc; Monnaie, Didier; Amicosante, Gianfranco; Pelicic, Vladimir; Gicquel, Brigitte; Frere, Jean-Marie; Galleni, Moreno
CS Centre d'Ingenierie des Proteines, Universite de Liege, Institut de Chimie (B6), Sart Tilman, Liege, B-4000/1, Belg.
SO FEBS Letters (1997), 406(3), 275-278
CODEN: FEBLAL; ISSN: 0014-5793
PB Elsevier
DT Journal
LA English
AB Mycobacterium fallax (M. fallax) is naturally sensitive to many .beta.-lactam antibiotics (MIC<2 .mu.g/mL) and devoid of .beta.-lactamase activity. In this paper, we show that the prodn. of the .beta.-lactamase of Mycobacterium fortuitum by M. fallax significantly increased the MIC values for good substrates of the enzyme, whereas the potency of poor substrates or transient inactivators was not modified. The rates of diffusion of .beta.-lactams through the mycolic acid layer were low, but for all studied compds. the half-equilibration times were such that they would only marginally affect the MIC values in the absence of .beta.-lactamase prodn. These results emphasize the importance of enzymic degrdn. as a major factor in the resistance of mycobacteria to penicillins.

L16 ANSWER 46 OF 65 USPATFULL on STN
AN 96:113834 USPATFULL
TI Bacterial expression vectors containing DNA encoding secretion signals of lipoproteins
IN Stover, Charles K., Silver Spring, MD, United States
PA MedImmune, Inc., Gaithersburg, MD, United States (U.S. corporation)
PI US 5583038 19961210
AI US 1992-977630 19921117 (7)
RLI Continuation-in-part of Ser. No. US 1991-780261, filed on 21 Oct 1991, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Fleisher, Mindy; Assistant Examiner: Carter, Philip W.
LREP Olstein, Elliot M.
CLMN Number of Claims: 31
ECL Exemplary Claim: 1
DRWN 60 Drawing Figure(s); 64 Drawing Page(s)
LN.CNT 2112
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB An expression vector for expressing a protein or polypeptide in a bacterium, which comprises a first DNA sequence encoding at least a secretion signal of a lipoprotein, and a second DNA sequence encoding a protein or fragment thereof, or polypeptide or peptide heterologous to the bacterium which expresses the protein or fragment thereof, or polypeptide or peptide. The bacterium expresses a fusion protein a lipoprotein or lipoprotein segment and the protein or fragment thereof, or polypeptide or peptide heterologous to the bacterium which expresses the protein or fragment thereof, or polypeptide or peptide. Such expression vectors increase the immunogenicity of the protein or

fragment thereof, or polypeptide or peptide by enabling the protein or fragment thereof, or polypeptide or peptide to be expressed on the surface of the bacterium. Bacteria which may be transformed with the expression vector include mycobacteria such as BCG. The expression vectors of the present invention may be employed in the formation of live bacterial vaccines against Lyme disease wherein the bacteria express a surface protein of *Borrelia burgdorferi*, the causative agent of Lyme disease.

L16 ANSWER 47 OF 65 USPATFULL on STN

AN 96:27116 USPATFULL

TI Recombinant mycobacterial vaccine

IN Bloom, Barry R., Hastings on Hudson, NY, United States

Davis, Ronald W., Palo Alto, CA, United States

Jacobs, Jr., William R., Bronx, NY, United States

Young, Richard A., Winchester, MA, United States

Husson, Robert N., Takoma Park, MD, United States

PA Albert Einstein College of Medicine of Yeshiva University, Bronx, NY, United States (U.S. corporation)

The Board of Trustees of the Leland Stanford, Jr. University, Stanford, CA, United States (U.S. corporation)

Whitehead Institute for Biomedical Research, Cambridge, MA, United States (U.S. corporation)

PI US 5504005 19960402

AI US 1989-361944 19890605 (7)

RLI Continuation-in-part of Ser. No. US 1988-223089, filed on 22 Jul 1988, now abandoned And Ser. No. US 1988-216390, filed on 7 Jul 1988, now abandoned, each which is a continuation-in-part of Ser. No. US 1988-163546, filed on 3 Mar 1988, now abandoned which is a continuation-in-part of Ser. No. US 1987-20451, filed on 2 Mar 1987, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Stone, Jacqueline; Assistant Examiner: LeGuyader, J.

LREP Hamilton, Brook, Smith & Reynolds

CLMN Number of Claims: 29

ECL Exemplary Claim: 1

DRWN 23 Drawing Figure(s); 17 Drawing Page(s)

LN.CNT 2391

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Recombinant mycobacterial vaccine vehicles capable of expressing DNA of interest which encodes at least one protein antigen for at least one pathogen against which an immune response is desired and which can be incorporated into the mycobacteria or stably integrated into the mycobacterial genome. The vaccine vehicles are useful for administration to mammalian hosts for purposes of immunization. A recombinant vector which replicates in *E. coli* but not in mycobacteria is also disclosed. The recombinant vector includes 1) a mycobacterial gene or portions thereof, necessary for recombination with homologous sequences in the genome of ***mycobacteria*** ***transformed*** with the recombinant plasmid; 2) all or a portion of a gene which encodes a polypeptide or protein whose expression is desired in ***mycobacteria*** ***transformed*** with the recombinant plasmid;

3) DNA sequences necessary for replication and selection in *E. coli*; and

4) DNA sequences necessary for selection in mycobacteria (e.g., drug resistance). The present invention also relates to two types of

recombinant vectors useful in introducing DNA of interest into mycobacteria, where it is expressed. One type of vector is a recombinant phasmid capable of replicating as a plasmid in E. coli and of lysogenizing a mycobacterial host. The other type of vector is a recombinant plasmid which can be introduced into mycobacteria, where it is stably maintained extrachromosomally.

L16 ANSWER 48 OF 65 LIFESCI COPYRIGHT 2003 CSA on STN
 AN 97:62999 LIFESCI
 TI Recombinant mycobacterial vaccine
 CS YESHIVA UNIVERSITY
 SO (1996) . US Patent 5504005; US Cl. 435/253.1 435/69.1 435/69.3 435/69.51
 435/69.52 435/172.1 435/172.3 435/183 435/189 435/207 435/252.33
 435/320.1.
 DT Patent
 FS W3; A
 LA English
 AB Recombinant mycobacterial vaccine vehicles capable of expressing DNA of interest which encodes at least one protein antigen for at least one pathogen against which an immune response is desired and which can be incorporated into the mycobacteria or stably integrated into the mycobacterial genome. The vaccine vehicles are useful for administration to mammalian hosts for purposes of immunization. A recombinant vector which replicates in E. coli but not in mycobacteria is also disclosed. The recombinant vector includes 1) a mycobacterial gene or portions thereof, necessary for recombination with homologous sequences in the genome of ***mycobacteria*** ***transformed*** with the recombinant plasmid; 2) all or a portion of a gene which encodes a polypeptide or protein whose expression is desired in ***mycobacteria*** ***transformed*** with the recombinant plasmid; 3) DNA sequences necessary for replication and selection in E. coli; and 4) DNA sequences necessary for selection in mycobacteria (e.g., drug resistance). The present invention also relates to two types of recombinant vectors useful in introducing DNA of interest into mycobacteria, where it is expressed. One type of vector is a recombinant phasmid capable of replicating as a plasmid in E. coli and of lysogenizing a mycobacterial host. The other type of vector is a recombinant plasmid which can be introduced into mycobacteria, where it is stably maintained extrachromosomally.

L16 ANSWER 49 OF 65 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED.
 on STN DUPLICATE 8
 AN 96008760 EMBASE
 DN 1996008760
 TI Genetic transformation of mycobacteria by homologous recombination.
 AU Mustafa A.S.
 CS Department of Microbiology, Faculty of Medicine, Kuwait University, P.O. Box 24923, Safat 13110, Kuwait
 SO Nutrition, (1995) 11/5 SUPPL. (670-673).
 ISSN: 0899-9007 CODEN: NUTRER
 CY United States
 DT Journal; Conference Article
 FS 004 Microbiology
 026 Immunology, Serology and Transplantation
 037 Drug Literature Index
 LA English
 SL English
 AB Mycobacteria are highly potent adjuvants; therefore, expression of foreign

genes in mycobacteria provides a delivery system to induce strong immune responses against foreign proteins. In this study we report transformation of *Mycobacterium smegmatis* by homologous recombination using pUC19-based plasmid vectors with *pyrF* gene of *M. smegmatis* (pY6001) or *pyrF* gene disrupted by introducing the aminoglycoside phosphotransferase (*aph*) gene (pY6002). Both of these plasmids were used to transform the host cells by electroporation. The transformation and selection conditions were optimized with respect to cell number, stage of cell growth, DNA concentration, postelectroporation incubation time, and kanamycin concentration. With the plasmid Y6002, the transformation was usually a result of single crossover (class I transformants) and only 5% transformants were generated by double crossover (class II transformants). The double crossover led to the replacement of wild-type *pyrF* gene with the *aph*-disrupted *pyrF* gene. The gene replacement could also occur by resolution of the class I transformants into class II, but at a very low frequency. Further experiments were done to determine if the wild-type genotype could be rescued by retransformation with pY6001. Similar transformation efficiencies, as reported above, were obtained, but the frequency of double crossover increased to 35%. This transformation strategy provides a way by which the ***mycobacteria***

transformed with foreign genes will not require drug selection, a trait preferred to develop recombinant vaccines.

L16 ANSWER 50 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1993:678728 CAPLUS
 DN 119:278728
 TI Prokaryotes including DNA encoding bacteriophage immunity
 IN Jacobs, William R.; Hatfull, Graham
 PA Albert Einstein College of Medicine, USA; University of Pittsburgh
 SO PCT Int. Appl., 47 pp.
 CODEN: PIXXD2
 DT Patent
 LA English
 FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9319603	A1	19931014	WO 1993-US2655	19930312
	W: AU, CA, FI, JP, NO				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	AU 9339297	A1	19931108	AU 1993-39297	19930312
PRAI	US 1992-861002		19920331		
	WO 1993-US2655		19930312		

AB A prokaryote, esp. a ***mycobacterium***, is ***transformed*** with DNA which includes a sequence encoding immunity to a lytic bacteriophage. Such transformed mycobacteria may be employed in vaccines, thereby eliminating the need for vaccines contg. mycobacteria having antibiotic resistance markers. Thus, a segment of the mycobacteriophage L5 genome (gene 71) was identified which conferred immunity to L5 superinfection by wild-type L5 phage in *Mycobacterium smegmatis*. A plasmid (pMH35) was constructed which contained gene 71, an integrase gene, and a gene encoding kanamycin resistance, and the kanamycin resistance gene was removed by site-specific recombination using resolvase. BCG organisms electroporated with pMH35 produced colonies immune to infection with phage D29.

L16 ANSWER 51 OF 65 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 9

AN 1993:342481 BIOSIS
 DN PREV199396039481
 TI Transformation with katG restores isoniazid sensitivity in Mycobacterium tuberculosis isolates resistant to a range of drug concentrations.
 AU Zhang, Ying; Garbe, Thomas; Young, Douglas (1)
 CS (1) MRC Tuberculosis Related Infections Unit, Hammersmith Hospital, Ducane Road, London W12 0HS UK
 SO Molecular Microbiology, (1993) Vol. 8, No. 3, pp. 521-524.
 ISSN: 0950-382X.
 DT Article
 LA English
 AB Isoniazid-resistant isolates of ***Mycobacterium*** tuberculosis were ***transformed*** with a plasmid vector carrying the functional catalase-peroxidase (katG) gene. Expression of katG restored full drug susceptibility in isolates initially resistant to concentrations ranging from 3.2 to gt 50 mu-g ml-1. Transformation with the corresponding katG gene from Escherichia coli resulted in low-level expression of catalase and peroxidase activities and conferred partial isoniazid sensitivity.

L16 ANSWER 52 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN
 AN 1992:201079 CAPLUS
 DN 116:201079
 TI Mycobacterial expression vector and transgenic mycobacteria for use as vaccines
 IN Jacobs, Paul; Haeseleer, Francoise; Massaer, Marc; Bollen, Alex
 PA SmithKline Beecham Biologicals S. A., Belg.
 SO PCT Int. Appl., 39 pp.
 CODEN: PIXXD2
 DT Patent
 LA English
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9201796	A1	19920206	WO 1991-EP1332	19910713
	W: AU, CA, JP, KR, US				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
	AU 9181012	A1	19920218	AU 1991-81012	19910713
	EP 544685	A1	19930609	EP 1991-912483	19910713
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	JP 06508741	T2	19941006	JP 1991-511864	19910713
PRAI	GB 1990-15888		19900719		
	WO 1991-EP1332		19910713		

AB Mycobacterial expression vectors contain the promoter and ribosomal binding site of the 64 kDa protein of M. bovis-BCG fused to a gene of interest. ***Mycobacteria*** ***transformed*** with such a vector can be used as vaccines (no data). Expression plasmids contg. the Plasmodium falciparum circumsporozoite protein gene fused to the 64 kDa protein gene regulatory sequences were prepd. and the chimeric genes were expressed in M. smegmatis and M. bovis-BCG. The circumsporozoite protein was expressed to the extent of 0.01-0.6% of the total cellular protein.

L16 ANSWER 53 OF 65 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 10
 AN 1991:482716 BIOSIS
 DN BA92:116476
 TI MULTIPLE FORMS OF O METHYLTRANSFERASE INVOLVED IN THE MICROBIAL CONVERSION OF ABIETIC ACID INTO METHYL ABIETATE BY MYCOBACTERIUM-SP.

AU ORPISZEWSKI J; HEBDA C; SZYKULA J; POWLS R; CLASPER S; REES H H
CS INST. ORGANIC PHYSICAL CHEM., TECHNICAL UNIVERSITY, WYSPIANSKIEGO 27,
50-370 WROCLAW, POLAND.
SO FEMS (FED EUR MICROBIOL SOC) MICROBIOL LETT, (1991) 82 (2), 233-236.
CODEN: FMLED7. ISSN: 0378-1097.
FS BA; OLD
LA English
AB Six out of seven tested strains of ***mycobacteria***
transformed abietic acid to methyl abietate in shake culture. The
conversion carried out by Mycobacterium sp. MB 3683 was induced by the
substrate and stimulated by methionine. Fractionation of the cell extract
of Mycobacterium sp. MB 3683 on DEAE cellulose, Ultrogel ACA 44 and MONO Q
resulted in the separation of three distinct methyltransferase activities
which could also esterify palmitic acid. The separated forms of the
methyltransferase exhibited different activities towards two substrates.

L16 ANSWER 54 OF 65 MEDLINE on STN
AN 92038933 MEDLINE
DN 92038933 PubMed ID: 1936951
TI Multiple forms of O-methyltransferase involved in the microbial conversion
of abietic acid into methyl abietate by Mycobacterium sp.
AU Orpiszewski J; Hebda C; Szykula J; Powls R; Clasper S; Rees H H
CS Institute of Organic and Physical Chemistry, Technical University,
Wroclaw, Poland.
SO FEMS MICROBIOLOGY LETTERS, (1991 Aug 1) 66 (2) 233-6.
Journal code: 7705721. ISSN: 0378-1097.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199112
ED Entered STN: 19920124
Last Updated on STN: 19970203
Entered Medline: 19911213
AB Six out of seven tested strains of ***mycobacteria***
transformed abietic acid to methyl abietate in shake culture.
The
conversion carried out by Mycobacterium sp. MB 3683 was induced by the
substrate and stimulated by methionine. Fractionation of the cell extract
of Mycobacterium sp. MB 3683 on DEAE cellulose, Ultrogel ACA 44 and MONO
Q resulted in the separation of three distinct methyltransferase
activities which could also esterify palmitic acid. The separated forms
of the methyltransferase exhibited different activities towards these two
substrates.

L16 ANSWER 55 OF 65 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AN 91:492821 SCISEARCH
GA The Genuine Article (R) Number: GD210
TI MULTIPLE FORMS OF O-METHYLTRANSFERASE INVOLVED IN THE MICROBIAL CONVERSION
OF ABIETIC ACID INTO METHYL ABIETATE BY MYCOBACTERIUM SP
AU ORPISZEWSKI J (Reprint); HEBDA C; SZYKULA J; POWLS R; CLASPER S; REES H H
CS WROCLAW TECH UNIV, INST ORGAN & PHYS CHEM, WYSPIANSKIEGO 27, PL-50370
WROCLAW, POLAND (Reprint); UNIV LIVERPOOL, DEPT BIOCHEM, LIVERPOOL L69
3BX, ENGLAND
CYA POLAND; ENGLAND
SO FEMS MICROBIOLOGY LETTERS, (1991) Vol. 82, No. 2, pp. 233-236.
DT Article; Journal

FS LIFE
LA ENGLISH
REC Reference Count: 6

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Six out of seven tested strains of ***mycobacteria***
transformed abietic acid to methyl abietate in shake culture. The conversion carried out by Mycobacterium sp. MB 3683 was induced by the substrate and stimulated by methionine. Fractionation of the cell extract of Mycobacterium sp. MB 3683 on DEAE cellulose, Ultrogel Aca 44 and MONO Q resulted in the separation of three distinct methyltransferase activities which could also esterify palmitic acid. The separated forms of the methyltransferase exhibited different activities towards these two substrates.

L16 ANSWER 56 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1989:2194 CAPLUS

DN 110:2194

TI Phasmids, and ***mycobacteria*** ***transformed*** with phasmids for use as vaccines

IN Bloom, Barry R.; Davis, Ronald W.; Jacobs, William R., Jr.; Young, Richard A.

PA Whitehead Institute for Biomedical Research, USA

SO PCT Int. Appl., 55 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 8

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 8806626	A1	19880907	WO 1988-US614	19880229
	W: JP				
	RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
	EP 347425	A1	19891227	EP 1988-903026	19880229
	EP 347425	B1	19951227		
	R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
	JP 02504461	T2	19901220	JP 1988-502787	19880229
	JP 3011939	B2	20000221		
	EP 681026	A1	19951108	EP 1995-201559	19880229
	R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
	AT 132195	E	19960115	AT 1988-903026	19880229
	JP 11335296	A2	19991207	JP 1999-77706	19880229
	CA 1336270	A1	19950711	CA 1988-560339	19880302
PRAI	US 1987-20451	A	19870302		
	EP 1988-903026	A3	19880229		
	JP 1988-502787	A3	19880229		
	WO 1988-US614	W	19880229		

AB Phasmids (shuttle vectors which replicate as a plasmid in bacteria and replicate as a phage in mycobacteria) for expression of foreign DNA in mycobacteria are constructed. Phasmids encoding .gtoreq.1 protein antigen are used to prep. mycobacterial vaccines. A mycobacteria transfection system allowing transfection frequencies of >105 pfu/.mu.g D29 DNA was developed. Phasmid phAE1 was prepd. by (1) digesting mycobacteriophage TM4 DNA with Sau3A to prep. 30-50 kb fragments which were inserted into cosmid pHC79; (2) DNA fragments of 38-52 kb contg. .lambda. COS sites were packaged into .lambda. heads in vitro, Escherichia coli was transduced with these phage, and ampicillin-resistant colonies were selected; (3) spheroplasts prepd. from TM4-infected Mycobacterium smegmatis were

transfected with these pHc79 derivs. to prep. TM4 phage with the pHc79 deriv. inserted into a non-essential region. The aph gene of TN903 (a 1.6 kb DNA fragment) was inserted into pHEA1). The resulting phasmid was successfully transfected into M. smegmatis.

L16 ANSWER 57 OF 65 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1989:16224 BIOSIS
DN BR36:3901
TI MORPHOLOGY OF CHANGES TUBERCLE BACILLI FORMS.
AU NIKOLAEVA G M; DOROZHKOVA I R
CS CENT. RES. INST. TUBERC., MINIST. HEALTH USSR, MOSCOW, USSR.
SO Probl. Tuberk., (1988) 0 (4), 57-59.
CODEN: PRTUAX. ISSN: 0032-9533.
FS BR; OLD
LA Russian

L16 ANSWER 58 OF 65 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1988:264291 BIOSIS
DN BA86:3535
TI PRODUCTION OF XYLITOL FROM D XYLULOSE BY MYCOBACTERIUM-SMEGMATIS.
AU IZUMORI K; TUZAKI K
CS DEP. BIORESOURCE SCI., FAC. AGRIC., KAGAWA UNIV., MIKI-CHO, KAGAWA 761-07, JPN.
SO J FERMENT TECHNOL, (1988) 66 (1), 33-36.
CODEN: JFTED8. ISSN: 0385-6380.
FS BA; OLD
LA English

AB ***Mycobacterium*** smegmatis ***transformed*** D-xylulose to xylitol in washed cell reactions under aerobic and anaerobic conditions. The yield of xylitol reached about 70% in anaerobic conditions (in N2) by cells grown on media containing xylitol or D-mannitol. Cells immobilized with Ca-alginate had almost the same activity of xylitol production as washed cells. Xylitol was produced from D-xylulose using commercial immobilized D-xylulose isomerase from Bacillus coagulans and immobilized cells of M. smegmatis. From 10 g of D-xylulose, 4 g of xylitol was produced and 5 g of D-xylulose remained in the reaction mixture; no D-xylulose was detected.

L16 ANSWER 59 OF 65 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 11
AN 1987:358946 BIOSIS
DN BA84:56349
TI EXPRESSION OF PROTEINS OF MYCOBACTERIUM-TUBERCULOSIS IN ESCHERICHIA-COLI AND POTENTIAL OF RECOMBINANT GENES AND PROTEINS FOR DEVELOPMENT OF DIAGNOSTIC REAGENTS.
AU COHEN M L; MAYER L W; RUMSCHLAG H S; YAKRUS M A; JONES W D JR; GOOD R C
CS DIV. BACTERIAL DISEASES, CENT. INFECTIOUS DISEASES, CENT. DISEASES CONTROL, ATLANTA, GA. 30333.
SO J CLIN MICROBIOL, (1987) 25 (7), 1176-1180.
CODEN: JCMIDW. ISSN: 0095-1137.
FS BA; OLD
LA English
AB Recombinant plasmids containing DNA from ***Mycobacterium*** tuberculosis were ***transformed*** into Escherichia coli, and three colonies were selected by their reactivity with polyclonal antisera to M. tuberculosis. The three recombinant vectors contained DNA inserts of different sizes flanking a common 4.7-kilobase (kb) sequence. Each

recombinant produced 35- and 53-kilodalton proteins (35K and 53K proteins, respectively) which were absent in the control *E. coli*. In Western blotting experiments, both proteins bound several antisera to *M. tuberculosis* but not antisera to other commonly isolated mycobacteria. Rabbits immunized with the recombinant 35K protein produced antisera which bound to both the 35K and 53K protein bands, a single 35K protein band present in a culture filtrate of *M. tuberculosis*, and single protein bands with differing molecular weights in whole-cell homogenates from other *Mycobacterium* spp. An additional recombinant vector containing a 2.2-kb subclone of the 4.7-kb sequence was constructed and, when used as a probe, demonstrated homology with various fragments of chromosomal digests of selected mycobacteria. Reactivity of this probe to *Mycobacterium bovis* and *M. bovis* BCG was indistinguishable from reactivity to *M. tuberculosis*. Immunoglobulin G reactivity to the 35K antigen was detected in antisera from 8 of 20 persons with active tuberculosis, 4 of 18 persons with leprosy, and none of 14 healthy controls. In contrast, reactivity to various proteins in *M. tuberculosis* culture filtrate was present in 18 of 20 patients with tuberculosis, 16 to 18 patients with leprosy, and 5 of 14 controls. The production of *M. tuberculosis* proteins by *E. coli* circumvents many difficulties encountered in the growth and manipulation of *M. tuberculosis* and may facilitate the development of better diagnostic and immunizing reagents.

L16 ANSWER 60 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1988:128222 CAPLUS

DN 108:128222

TI Stereochemical studies of biological alkylation reactions

AU Arigoni, Duilio

CS Eidgenoessische Tech. Hochsch., Lab. Org. Chem., Zurich, Fed. Rep. Ger.

SO Chimia (1987), 41(6), 188-9

CODEN: CHIMAD; ISSN: 0009-4293

DT Journal

LA German

AB The stereochem. mechanisms of 2 biol. alkylation reactions were studied. Using S-adenosylmethionine as a 1-C donor, ***Mycobacterium*** phlei ***transformed*** oleic acid to tuberculostearic acid via reductive alkylation and Lactobacillus plantarum transformed vaccenic acid to lactobacillic acid.

L16 ANSWER 61 OF 65 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 12

AN 1986:126451 BIOSIS

DN BA81:36867

TI STRUCTURAL DETERMINATION OF UNSATURATED MYCOLIC ACIDS BY FAST ATOM BOMBARDMENT AND TANDEM MASS SPECTROMETRY ANALYSES OF THEIR AMINO ALCOHOL DERIVATIVES.

AU RIVIERE M; CERVILLA M; PUZO G

CS CENT. RECH. BIOCHIM. GENETIQUE CELL. C.N.R.S., 118, ROUTE NARBONNE, 31062 TOULOUSE CEDEX, FR.

SO ANAL CHEM, (1985) 57 (13), 2444-2448.

CODEN: ANCHAM. ISSN: 0003-2700.

FS BA; OLD

LA English

AB The ethylenic functions of the mycolic acids isolated from ***Mycobacterium*** smegmatis were ***transformed*** into amino alcohols. Their analyses by positive fast atom bombardment mass spectrometry allows their molecular weight to be unambiguously established

from their pseudomolecular ions. Moreover their MIKE-CID (mass analyzed ion kinetic energy collision induced dissociation) mass spectra permit the amino groups borne by the aliphatic chain and consequently the ethylenic functions in the native molecule investigated to be located.

L16 ANSWER 62 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1979:484180 CAPLUS

DN 91:84180

TI Effect of bacteriocin from *Mycobacterium smegmatis* on the growth of cultured cells

AU Saito, Hajime; Watanabe, Takashi

CS Dep. Microbiol. and Immunol., Shimane Med. Univ., Shimane, Japan

SO Igaku to Seibutsugaku (1978), 96(6), 393-7

CODEN: IGSBAL; ISSN: 0019-1604

DT Journal

LA Japanese

AB Bacteriocin produced by *M. smegmatis* strain ATCC 14468 extensively inhibited the growth of virus-transformed cells in culture, compared with the effect on nontransformed cells.

L16 ANSWER 63 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1976:557877 CAPLUS

DN 85:157877

TI Transformed steroids. 81. Preparation of .DELTA.1,4-3-keto steroids using immobilized bacterial cells

AU Voishvillo, N. E.; Kamernitskii, N. E.; Khaikova, A. Ya.; Leont'ev, I. G.; Paukov, V. N.; Nakhapetyan, L. A.

CS Inst. Org. Khim. im. Zelinskogo, Moscow, USSR

SO Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya (1976), (6), 1357-60

CODEN: IASKA6; ISSN: 0002-3353

DT Journal

LA Russian

AB Several species of *Arthobacter*, *Mycobacterium*, and *Nocardia* immobilized in 7% polyacrylamide gel transformed .DELTA.5-3.beta.-acetoxy-, .DELTA.5-3.beta.-hydroxy- and .DELTA.4-3-ketosteroids into .DELTA.1,4-3-keto compds. by means of 1,2-dehydrogenation. 16.alpha.,17.alpha.-Isopropylidenedihydroxypregnenolone [14072-38-9] was transformed to its .DELTA.1,4-3-keto deriv. [5094-23-5]. The yield of isolated steroids from such a transformation reached 90-100%.

****Mycobacterium**** globiforme ***transformed***

17.alpha.-methylandrostenediol, pregnenolone, pregnenolone 3-acetate, and progesterone with 68-85% yield of .DELTA.1,4-3-ketones.

L16 ANSWER 64 OF 65 MEDLINE on STN

AN 76202605 MEDLINE

DN 76202605 PubMed ID: 818881

TI Metabolic fate of cholesteryl methyl ether in *Mycobacterium phlei*.

AU Buki K G; Ambrus G; Horvath G

SO ACTA MICROBIOLOGICA ACADEMIAE SCIENTIARUM HUNGARICAE, (1975) 22 (4) 447-51.

Journal code: 0370333. ISSN: 0001-6187.

CY Hungary

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 197608

ED Entered STN: 19900313

Last Updated on STN: 19900313

Entered Medline: 19760802

AB ***Mycobacterium*** phlei . ***transformed*** cholesteryl methyl ether into three metabolites: 3beta-methoxy-dinor-5,17(20)-choladien-22-oic methyl ester (I), 3beta-methoxy-5-androsten-17-one (II), and 3beta-methoxy-dinor-5-cholen-22-ol (III). After isolation with thin-layer chromatography, their structures were elucidated by mass, IR and NMR spectroscopy. Compound II was the major product. Compounds I and III were products of various side reactions. In the presence of 8-hydroxyquinoline that inhibits degradation of the steroid nucleus, 1,4-androstadiene-3,17-dione was formed in addition to the compounds mentioned. This indicates that a moderate splitting of the ether bond takes place.

L16 ANSWER 65 OF 65 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1967:418728 CAPLUS

DN 67:18728

TI Transformation of cholic acid with the aid of Mycobacterium 1210

AU Severina, L. O.; Torgov, I. V.; Skryabin, G. K.

CS Inst. Microbiology, Moscow, USSR

SO Doklady Akademii Nauk SSSR (1967), 173(5), 1200-2

CODEN: DANKAS; ISSN: 0002-3264

DT Journal

LA English

AB Uv, ir, P.M.R., and mass spectral evidence showed that incubation with ***Mycobacterium*** 1210 ***transformed*** cholic acid into 2 derivs., one satd. and the other unsatd. at position 8:9. Complex transformation of cholic acid also appeared to involve creation of a .DELTA.4-3-keto group on ring A, oxidn. of the 12-hydroxy group to a keto group, and .beta.-oxidn. of the side chain.